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A MICROCHEMICAL AND MORPHOLOGICAL STUDY OF THE DEVELOPING ENDOSPERM OF MAIZE[1]

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(WITH PLATE III AND SEVEN FIGURES)

Introduction

The endosperm of *Zea mays* L., as of other cereal grains, has been looked upon as a place of food storage with the special function of contributing materials to the embryo during development of the kernel as well as during its germination. No evidence has appeared in this study, however, to support the idea that the embryo brings about any digestion or makes any use whatever of the polysaccharides being stored in the living endosperm cells during the period of kernel development. On the contrary, the two structures develop simultaneously, and the endosperm appears as a competitor of the embryo, sharing the same receptacle and the same food supply. In its development, therefore, it appears to be as distinct an entity as the embryo.

The aim of this paper is to describe the development of the endosperm of the genetic types of corn, starchy, waxy, sweet, and waxy-sweet, in terms of cellular activity. The investigation was begun in 1924 in the botanical laboratories of Ohio State University, and later finished here in 1927. In the interim it was carried on at The Boyce Thompson Institute at Yonkers, New York. No attempt has been

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made to separate the findings made at the two institutions. The results reported are from the plantings of corn studied at the Institute in the summer of 1926.

Review of literature

Most of the ideas which have been advanced to explain the phylogenetic significance of the endosperm were early summarized by SARGANT (**25**). TRELEASE (**30**) has suggested that the endosperm be called the *xeniophyte*, since it may be regarded as a structure distinctly not forming a part of either the gametophyte or sporophyte generations, and since it first shows the occurrence of crossing where this is recognizable in the developing seed.

The origin of the endosperm of corn by triple fusion was established by GUIGNARD (**9**) in 1901, and was first illustrated in publications by WEATHERWAX (**32**) and MILLER (**20**). GUIGNARD also saw the coenocytic condition of the very young endosperm and the parietal position of the free nuclei in the embryo sac. WEATHERWAX (**34**) described the centripetal wall formation, which was found to begin when about 50 free nuclei are present and to continue until the endosperm is a complete cellular body. The early development and shape of the tissue were recorded for starchy corn by POINDEXTER (**24**), whose figures show that the embryo is surrounded at the beginning by the endosperm and that it is laterally placed at the dorsal side of the tissue. FISK (**8**) noted the poorly restricted cambial activity in the very young endosperm. MOTTIER (**22**) described the more specific cambial region present in the cap or crown of the kernel in both a sweet and a starchy type of corn during the milk stage in development, and followed the appearance and differentiation of the aleurone layer during the same period. MANGELSDORF (**18**) reported small starch grains as occurring in the upper cells of the endosperm at the early milk stage, 15–20 days after pollination. By the time the late milk stage was reached, 25–28 days after pollination, he found that the cells in the upper part of the endosperm were completely packed with starch grains, although those in the lower part were still relatively clear. His paper includes microphotographs of sectioned normal kernels at the younger stages mentioned.

That the endosperm of corn has a specific ontogeny has long been

recognized. Calculations from the records of KIESSELBACH (**14**) for starchy corn grown during midsummer field conditions in Nebraska from 1911 to 1921 show an average growth period of approximately 55 days for the whole kernel. APPLEMAN and EATON (**1**) and CULPEPPER and MAGOON (**6**) have demonstrated that the rate of growth of the kernel, and hence the growing period of the endosperm, is measurably affected by environmental conditions, particularly temperature. LAMPE (**15**) found that rate of growth of the kernel under different temperature conditions is expressed in the regional progress of growth in the endosperm, and in the consequent distribution of carbohydrates.

Data showing the effect of maturity upon yield are indirectly of value in determining when polysaccharide storage in the endosperm actually ceases to occur. As early as 1889, SHELTON (**26**) measured the increase in bushels per acre for several starchy varieties of corn, harvesting at four cardinal points in the development of the kernel. He showed that corn allowed to mature completely on the stalk continues to increase in yield for some time after it is usually cut, the increase being as great as 10–12 per cent after the husks are dry and the leaves are turning, which was recorded as the usual stage in Kansas for cutting the crop. Similar measurements were made recently by THATCHER (**29**) at Wooster, Ohio, where the weekly increments in acre yield, measured in bushels of shelled corn harvested and in test weight per bushel, indicate an increase in yield proportional to the degree of maturity attained by the crop.

During the last 75 years many chemical analyses have been made of the mature kernels. Those of PEARL and BARTLETT (**23**) on starchy and sweet corn, and of JONES (**12**) on starchy, pseudostarchy, and sweet types, made to demonstrate the effects of Mendelian segregation, are representative of the results obtainable. HOPKINS, SMITH, and EAST (**10**) carried out detailed chemical analyses of the different parts of mature kernels of starchy corn, and STRAUGHN (**28**) a similar but more limited examination of those of sweet corn.

Analyses mainly of the kernels of sweet varieties at or near the edible stage have also been made by many workers. CULPEPPER and MAGOON (**5, 6**) were the first to make extensive periodic studies of

developing kernels of both sweet and starchy types. Their analyses were carried out on samples taken 5–30 days after pollination, and in the case of later plantings, up to 60 days, when an approximate equivalent of the 30-day kernel was found. The work of these men is of further importance since their analyses show reducing and non-reducing sugars, water-soluble and total polysaccharides. The amount of water-soluble polysaccharide is also evident indirectly in the results of JONES (**12**).

Studies of (1) the nature of the polysaccharides synthesized in the endosperm of sweet corn, (2) the nature and plastid origin of the units elaborated, and (3) the determining hereditary allelomorphs, were summarized by LAMPE and MEYERS (**16**) in 1925. Recently BRINK (**3**) has published data on the chemical properties of the polysaccharide in the endosperm of the waxy type which was originally discovered by WEATHERWAX (**33**). A bibliography comprising a great variety of papers was assembled by WEATHERWAX (**34**), and many references to chemical studies have been listed by CULPEPPER and MAGOON (**5**).

Production of experimental material

In studying the progressive development of the endosperm of corn, it is necessary to begin with healthy vigorous plants which under suitable growing conditions will furnish it with a constant abundant food supply. On the basis of their appearance, the plants used fulfilled these requirements.

CONDITIONS OF GROWTH.—The results reported in the present paper, except where indicated, were obtained from plants grown at the Boyce Thompson Institute at Yonkers, New York, during the summer of 1926. The plots were situated on well drained upland loam soil.[2] The rows were spaced at 3 feet, and the seeds planted 18 inches apart in the row. Two plantings were made from each lot of seed, the first on May 13 and the second on June 8. Cultivation was done by hoeing.

[2] The soil was described by GUY W. CONREY of the Department of Soils of Ohio State University: as (1) Gloucester loam having a brown color with a grayish cast; (2) situated near the highest point in an upland on the east bank of the Hudson River at an elevation of 320–340 feet above sea level; and (3) nearly level, sloping slightly to the southeast. See soil map, White Plains, New York area, 1919.

SEED.—The starchy type included Burr-Leaming dent and a flint variety. The former was from a double cross of inbred lines of the dent type originally developed by JONES (13) at the Connecticut Agricultural Experiment Station. The latter was an F_2 inbred generation from a cross between inbred lines from two varieties of flint corn. The seed of the waxy type was an F_1 generation from a cross between inbred lines homozygous for the waxy factor. The seed of the sweet type was from reciprocal crosses between two good quality inbred strains of the Golden Bantam variety. The Jones pseudo-starchy seed was of a relatively pure inbred line of sweet corn which was originally selected by JONES (12) for its pseudo-starchy character. The waxy-sweet type was represented by an F_4 inbred generation of a segregate homozygous for the recessive allelomorphs *su wx*, originally obtained from a cross between waxy and ordinary sweet corn. All the seed was from plants grown in 1924 or 1925.

SEASONAL DEVELOPMENT.—The growing season of 1926 at Yonkers was somewhat unusual, in that July and the early part of August were exceedingly warm, dry, and with but few days of cloudy weather. During this period the watering system in the garden was used frequently on the plants. Later, however, there was much rain and consecutively cloudy days.

The progressive development of the plants is of interest in this study only as it affects that of the endosperm, and observations made previous to pollination were not recorded. At pollination certain differences characteristic of the varieties studied were apparent. The Jones pseudo-starchy sweet and Burr-Leaming dent were free from suckers, although the former averaged 4 feet in height and the latter 8.5 feet. Golden Bantam sweet and flint had from one to three suckers, the former averaging 5 feet in height, and the latter 6.5 feet. Waxy and waxy-sweet had four or five suckers to the plant, the former being about 7 feet high and the latter 6 feet. Varietal differences were apparent in the length of the ear shoots. By tests made on the internode of the stalk situated above the ear, and on the shank at the second internode from the base, it was found during the time samples were taken that the stalk and shank in all the varieties were succulent and that the juice was very sweet. The amount and sweetness of the juice were less in the last

three collections of Golden Bantam, which were made 45–60 days after pollination. Even at 60 days, however, a moderate amount of sugar was present, as indicated by taste and by the osazone test.

During growth, the small leaves at the base of the stems of all the plants, which had appeared in the earlier stages of development, progressively died away. The upper leaves were uniformly succulent and green during the period of study, however, except those of Golden Bantam when the last three collections were taken. In this variety a gradual dying back from the tassel was noted, which appeared at the edges of the upper leaves at 40 days after pollination, in all the leaves at 50 days; and at 60 days the uppermost internode of the stalk and all the leaves were dead. The rest of the stalk was alive and greenish, and the leaves and stalk of the one sucker were also moderately succulent.

There was a progressive dying back of the husks in all the plants, which usually reached the middle of the ear at 35–40 days after pollination. The husks of Golden Bantam and flint were respectively dry and yellowish at the time the 50- and 55-day collections were made. An occasional plant became infected with smut and was removed from the garden as soon as noticed.

EVALUATION OF MATERIAL.—The first planting furnished all the collections used from the Jones pseudo-starchy and Golden Bantam sweet corn, and the last collection only, of the flint, waxy, and waxy-sweet types. The second planting was otherwise used as the source of material of the flint, Burr-Leaming dent, waxy, and waxy-sweet types.

Differences in the rates of development of the endosperm in the types studied were not determined for the same set of conditions because the plants did not reach the stage of pollination at the same time, and effects of differing environmental conditions were apparent. The periods of pollination of the plants used from the different types were as follows: flint, August 7–11; Burr-Leaming dent, August 15–17; waxy, August 17–20; Golden Bantam sweet, July 24–28; Jones pseudo-starchy, August 4–11; waxy-sweet, August 17. The last collection, made from the three types flint, waxy, and waxy-sweet, was from ears pollinated earlier, the first on July 28, and the other two on August 11. The number of days per sample between

the pollination and that of collection, therefore, were not equivalent in the amount of growth accomplished. Differences in vigor are also known to occur between inbred and crossbred strains. The general trend in development of the endosperm was nevertheless apparent, and it is to this phase of the problem that the present study is devoted.

Collection and handling of material

AGE OF SAMPLE.—WEATHERWAX (**32**) found in a variety of sweet corn in early September that about 24 hours elapsed between the time of pollination and that of fertilization. BUCHHOLZ and BLAKESLEE (**4**) have shown that under the usual conditions of heredity and environment, growth of pollen tubes in *Datura* is nearly uniform. Accordingly the age of the kernels was counted in days from the time of pollination to that of collection, no deduction being made for the time required for pollen tube growth. CULPEPPER and MAGOON (**5, 6**) had previously calculated the age of the kernels on open-pollinated ears in the same way.

The plants were hand-pollinated because cross-pollination would have occurred, and because kernels of known age were desired. Pollinations were made after the method employed by corn geneticists, so timed that ears filled with kernels to within an inch or two of the tip were obtained. All the ear shoots on a stalk were bagged until the time when the two upper ears were pollinated. The pedigree and the day and hour of pollination were recorded.

SCHEDULE OF SAMPLING.—During the most active part of the growing period, a study of the kernels in each variety was made every fifth day, and near the middle of each 5-day period. As the season advanced one sample every 5 days was taken. At longer intervals the whole plant was brought to the laboratory, when progress in development of the stalk, leaves, and ear shoot was observed. Observations and preparations from samples were usually carried out between 4 o'clock in the afternoon and 11 o'clock in the evening. The four earliest stages of Golden Bantam sweet corn, however, which were taken in the middle of the day, and three morning collections from other varieties, showed no significant difference in results because of the time of collection.

PROCEDURE IN SAMPLING.—Samples were taken at the time the

observations and preparations were to be made, the record sheets and reagents having been previously prepared. Only the upper ear of a plant was studied. One-half of the ear was collected at a time, and placed in a labeled bag. The husks and ear-bag were replaced over the other half of the ear. It had been shown by APPLEMAN and EATON (1) that the rate of development of the remaining kernels is not measurably affected by the removal of part of the ear if the husks are closed and held in place. The results of the writer during the previous season were in agreement, but the lower half of the ear was collected only when necessary. Two samples were usually taken at one time, the second being kept in a moist chamber in the ice box while the first was being handled. The kernels nearest the middle of the ear were studied when the upper half of it was collected, although the outer 1 inch was discarded when the lower half was used.

The order of observations, preparations, and recording was uniform for all samples, and was governed by the rate at which specific changes were likely to occur in the material. The record of the taste of the stalk and shank when these were tested, and the preparations for reducing and total sugars in the kernel were always taken care of first. The rest of the study followed in the order found to be most efficient. The procedure for one sample required less than 1 hour. Moisture samples from the late afternoon collections were afterwards prepared late at night, and those from the evening collections the next morning. During the interim the portions of the ears furnishing these samples were kept in a moist chamber in the ice box. There was no discernible change during this period in the kernels used.

Microchemical methods

In the chemical study it was not intended to find additional proofs for substances long accepted as present in the endosperm of corn, nor to search for those unidentified or reported only in traces. The aim was to find the localization of substances present in abundance, by a few reliable tests, for the bearing that the results would have upon the interpretation of endosperm development.

CUTTING AND WASHING SECTIONS.—Freehand sections cut longitudinally through the center of the kernel and the embryo were used. A small cavity, apparently resulting from tissue tensions, was usual-

ly found in the center of the older endosperms, and was therefore often present in the sections (fig. 11 *c*). For plastid and polysaccharide study the sections were about two cells thick, and for the sugar tests about 0.5 mm. thick. The depth of the latter provided many uncut cells and accentuated the localization of the resulting crystals. All sections were washed by twirling them rapidly in a Syracuse watch glass half-filled with distilled water. Forty revolutions requiring about 15 seconds were found to be satisfactory. The water was poured off immediately and the sections drained on clean filter paper. They were then transferred to the reagent desired. Washing was necessary since plastids and sap came freely out of the large cut cells, and good localization of materials was otherwise impossible. Even then critical observations were always made on uncut cells.

IODINE REAGENT.—An iodine solution, consisting of 0.3 gm. of iodine and 1.5 gm. of potassium iodide in 200 cc. of distilled water, was used in the study of plastid development and the consequent distribution of polysaccharides. Tests identifying the substances stored by the plastids as carbohydrates had been made earlier by LAMPE (**16**), and for the sake of speed in observing, the iodine reagent alone was used. The dilute solution neither distorted nor disintegrated the cell contents during the period of observation, yet the polysaccharide was sufficiently stained. By its use the desiccated globules were shown to be still intact in mature sweet corn, a fact not suspected at the publication of the preliminary paper (**16**), when the stronger reagent was being used.

A solution of twice the strength just noted was employed in making gross observations on sections and cut kernels, because of the more intense coloration produced. It was particularly useful in finding the extent of the core of globule-containing cells in sweet corn. This reagent was found to darken the protein content of the endosperm, and after some time to cause swelling of the grains of carbohydrate, especially in the younger kernels, and to disintegrate the compound grains and globules in the sweet varieties. The water medium and the pressure of the cover glass at the drying down of the mount also appeared to be factors in disintegration of the compound grains. When the current observations were completed, sections of

the kernels stained with iodine were remounted in levulose syrup (5 parts levulose; 3 parts water) and stored. The stain gradually faded out, although distribution of the solid carbohydrate was still evident.

POLARISCOPE.—This instrument had been used earlier (**16**) in the study of the bodies elaborated by the plastids. It was found also to be of value in the study of the decreasing moisture content of the kernel, as the grains of starch and waxy starch did not show the characteristic cross in the dark field until their moisture content was lowered either artificially or in the natural process of kernel maturation.

REDUCING AND NON-REDUCING SUBSTANCES IN KERNEL.—WEHMER (**35**) has listed glucose or invert sugar, saccharose, dextrin, and starch as having been reported in quantity in the corn kernel, and mentions tannin and pentosans, all of which have reducing power, either before or after acid inversion. VER HULST (**31**) reported 0.18 of 1 per cent free pentoses and 6.4 per cent pentosans in the kernels of starchy corn at the dent stage. Because only the reducing sugars, sucrose, dextrin, and starch are considered in the great bulk of quantitative work reported by other workers, and because of the specificity of the tests employed, these substances will be mentioned in the interpretation of the tests used in the present study.

INVERSION OF SUCROSE.—Invertase prepared in purified liquid form by Dr. H. R. KRAYBILL was used for inversion of the sucrose. It gave very satisfactory results when used with the phenylhydrazine reagent. The sections of the kernels were mounted in the undiluted invertase solution and placed on a warm plate kept at 45° C. until the excess moisture evaporated. While they were still moist the phenylhydrazine reagent and cover glasses were added.

OSAZONE TEST FOR REDUCING SUGARS.—A solution was made from two preparations: (1) phenylhydrazine hydrochloride in glycerin (1:10) and (2) sodium acetate in glycerin (1:10), which were mixed in the proportion of 1:2 at the time of using. Ten median sections from kernels on different sides of the ear were selected and the embryos removed. The remains of the kernels and the embryos were assembled separately in two rows on a 2×3 inch slide, and grouped conveniently for covering. The reagent was added directly to the

sections in the upper row, and after treatment with invertase to the lower. When the mounts were completed the slide was put on the warm plate (45° C.) and left for approximately 20 hours. The use of low temperatures in the study of sugars has been recommended by IRVINE (11), and the time of heating was found to be satisfactory in the plan of work followed. At the end of this period the rosettes of large crystals characteristic of the osazones of reducing sugars were striking. The crystals were well localized, the upper row of sections indicating the amount of reducing sugars present, and the lower the amount of total sugars, and hence, sucrose by difference. All the slides were examined and later placed in slide boxes which were stored in an upright position to prevent slipping of the mounts. MANGHAM (19) has noted that such preparations keep indefinitely when out of contact with air. The mounts prepared by the writer, although unsealed, showed no change in amount of precipitate present during the 8 months the slides were under frequent observation. Even now, 3 years later, the amount and distribution of crystallization are apparently unaltered, although fresh reagent has been added a few times to replace that lost by evaporation.

COPPER-REDUCTION TEST FOR REDUCING SUGARS.—Fluckiger's reagent, a sky-blue solution of copper tartrate powder in 20 per cent sodium hydroxide, which was made up at the time of using, was employed to a slight extent. The interpretation of this test is known to be governed (1) by the reducing substance present, (2) by the relative amounts of sample and reagent mixed together, (3) by the temperature of the mount, and (4) by the time of standing previous to observation. Uniformly small amounts of the pure sugars were tested separately. Fructose appeared to produce the maximum amount of cuprous oxide precipitate in 7 minutes at room temperature, and glucose, in 3 minutes at 45° C. Using commercial dextrin, the precipitate obtained in 5–20 minutes at 45° C. was considered to be a measure of the reducing power of the dextrin present. In the study of corn, however, the amount of sugar in the younger kernels was so great that satisfactory localization was not possible with this reagent. It also formed a white gel with the invertase solution and its use as a general test was abandoned.

SENSITIVITY OF SUGAR TESTS.—SIMON (27) states that Fehling's

solution, a copper-reduction test for reducing sugars, is sensitive to 0.0008 of 1 per cent, and that the osazone test is sensitive to 0.5–0.025 of 1 per cent. The relative sensitivity of the two types of reagents is shown in fig. 10 *b*, where the results of five tests are shown, three with Fluckiger's reagent (which is similar in reaction to Fehling's solution) and two with the phenylhydrazine reagent. One of the last two mounts was inverted. Before inversion the endosperm gave no crystals with the phenylhydrazine reagent, and only a small

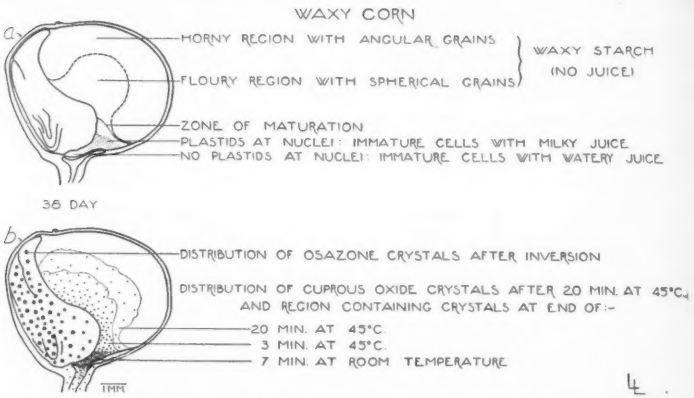
WAXY CORN
a
HORNY REGION WITH ANGULAR GRAINS
FLOURY REGION WITH SPHERICAL GRAINS
WAXY STARCH (NO JUICE)
ZONE OF MATURATION
PLASTIDS AT NUCLEI: IMMATURE CELLS WITH MILKY JUICE
NO PLASTIDS AT NUCLEI: IMMATURE CELLS WITH WATERY JUICE
38 DAY
b
DISTRIBUTION OF OSAZONE CRYSTALS AFTER INVERSION
DISTRIBUTION OF CUPROUS OXIDE CRYSTALS AFTER 20 MIN. AT 45°C. AND REGION CONTAINING CRYSTALS AT END OF:-
20 MIN. AT 45°C.
3 MIN. AT 45°C.
7 MIN. AT ROOM TEMPERATURE
TMR
4

FIG. 10.—Longitudinal sections of kernels of waxy corn 38 days after pollination, showing: *a*, position of gross internal regions of kernel and regional progress in cellular development and maturation; *b*, results of five tests for reducing and non-reducing substances. Amount and distribution of reducing substances indicated in endosperm, and that of non-reducing substance (sucrose) in embryo; minute amount of cuprous oxide precipitate obtained in hypocotyl of embryo not represented.

amount of precipitate with Fluckiger's in the basal part. The results obtained may be accounted for on the basis of the sensitivity of the two reagents, and indicate the presence of a small amount of reducing substance. The scarcity of non-reducing substance is apparent since the osazone test made after inversion again produced no crystals.[3] Previous to inversion the embryo gave negative results with the phenylhydrazine reagent, and only a minute amount of precipitate with Fluckiger's, in the region of the hypocotyl. The results

[3] The ear furnishing these kernels was collected in the late afternoon, March 20, 1926, from a plant growing in the greenhouse. It was examined as to general external

indicate the virtual absence of reducing substances. An abundance of non-reducing substance was present, however, since the osazone test made after inversion gave a heavy precipitate.

Because the amount of reducing substances other than the hexose sugars has not been found to be significant in the kernels of this species, and because there were no indications of a lack of penetration nor any interference with the reaction of the phenylhydrazine reagent or of the invertase, positive tests have been used to indicate the presence of reducing sugars, and negative tests to indicate their absence. The lower sensitivity of this reagent should be kept in mind, however, when the average distribution of sugars based on results with it is made.

PREPARATION OF MOISTURE SAMPLES.—The procedure employed by plant chemists was followed, except that a definite number of whole kernels was used. Each was freed from glumes and peduncle, and placed uninjured in the weighing bottle, the lid being lifted each time. The preliminary drying was done at 58° C. in an electric oven and the final drying at 100° C. in a vacuum oven. From these samples the percentages of moisture and dry matter in the kernels were obtained.

EVALUATION OF METHODS.—The microchemical methods recommended by ECKERSON (7) were modified with her help to suit the material used. The results agreed with the quantitative data obtained macrochemically by other workers, and an understanding of the development of the endosperm was made more nearly possible by fitting together the data made available by the two methods. Individual differences in samples and in localization of materials, which are usually covered up in macrochemical analysis by averages of determinations made on large samples, were detected microchemically.

Results from both methods have been of value in this study, in that those obtained macrochemically furnish a basis for quantitative estimation of the ones arrived at microchemically. On the other

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and internal appearance, then stored in a moist chamber in the ice box. The kernels were examined microscopically and for sugars on the following afternoon. The delay was probably responsible for the unusually low amount of sugars present in the base of the endosperm.

hand, the data obtained microchemically interpret and build on to those resulting from macrochemical analysis. The percentage of moisture in the kernels studied makes possible the comparison of results obtained in the present investigation with the quantitative data recorded by other workers. The book of methods of the Association of Official Agricultural Chemists (**2**) was used in checking the relationships apparent in the results obtained microchemically and macrochemically.

Results[4]

1. ONTOGENY OF AMYLIFEROUS CELL IN ENDOSPERM.—The following description applies only to the cells composing the major portion of the endosperm, not to those of the aleurone layer, nor to the peripheral glandlike cells situated in the basal portion of the endosperm above the vascular plate.

Starting with a very young cell in the endosperm, there was always a certain amount of enlargement, not only of the cell as a whole but of its nucleus. Development of vacuoles and increase in cell size were most prominent in those cells situated in the central portion of the endosperm. The cell walls remained thin and apparently undifferentiated. In general the nucleus was suspended in the vacuole by strands of granular cytoplasm, which showed streaming in the younger cells.

In the early studies (**16**) it became apparent that the larger granules in the cytoplasm developed into plastids which were responsible for the storage of polysaccharides. A definite grouping of the plastids about the nuclei was found to occur shortly before and at the beginning of storage. At this time differentiation in the plastids characteristic of the four types of corn (starchy, waxy, sweet, and waxy-sweet) was evident. The timing of events in the early growth of a

[4] A synopsis of the results recorded in this paper was presented before the Physiological Section of the Botanical Society of America, Philadelphia, December, 1927.

At the completion of this manuscript a paper appeared which also deals with the development of the endosperm of corn (WEATHERWAX, PAUL, The endosperm of *Zea* and *Coix*. Amer. Jour. Bot. **17**:371–380. 1930). The two papers agree on the following points: (1) the food supply of the older endosperm enters through its base; (2) there is an orderly development of the endosperm and a relatively high degree of differentiation of parts. WEATHERWAX also confirms the earlier report of the writer (**15**) as to the regional deposition of polysaccharides in the developing endosperm.

cell in the endosperm, as well as the changes which occur in it later, are given below. The rate of development described is that characteristic of healthy plants growing under the midsummer field conditions of 1926, at the Boyce Thompson Institute, Yonkers, New York.

The larger cells in the central part of an endosperm 5 days after pollination contained granules scattered throughout the cytoplasm, many of which were protoplastids (fig. 1). In the more advanced cells of a 7–10-day endosperm the developing plastids were grouped about the nuclei (figs. 2, 3). At about the tenth day, polysaccharide storage began in the cells in the upper central part of the endosperm (fig. 3). By about the twelfth day, the plastids in the more advanced cells were no longer grouped about the nuclei but were scattered within the cytoplasm (fig. 4).

As the plastids increased in size, the cytoplasmic network in which they were imbedded practically filled the cell, and the vacuole was relatively reduced and no longer obvious. In so far as noted, the plastid population of an individual cell developed uniformly and at about the same time. A small amount of variation in the size of the grains occurred during the period of development, however, and afterwards in the mature kernel.

A study of plastid development was made the previous season, using waxy corn pollinated August 15, 1925. Measurements of the grains of polysaccharide in the most advanced cells of the endosperm were made daily. They increased steadily in size from 1.5 μ at 10.5 days after pollination, when polysaccharide storage in the endosperm began, to 18.2 μ at 30 days. Subsequently no significant increase in their size could be detected, and further discernible changes in the cells occurred only at the drying down of the kernel. When the maximum size of the grains of carbohydrates was reached, they were closely packed and angular in the region of the endosperm which later became horny; they were loosely packed and nearly spherical in the region which matured floury. The distribution of grains of the two shapes is shown in the fig. 10 *a*, which, however, represents a kernel after drying down had begun.

2. ONTOGENY OF ENDOSPERM.—What was hitherto known of the development of the endosperm is in the main summarized in the

review of literature, and a sufficient presentation of the initiation of the endosperm may be found there. Collections in the present study began 5 days after pollination.

The very young endosperm, 5–8 days after pollination, was made up of cells much alike in appearance, except (as noted by FISK 8) that the peripheral ones were smaller and less vacuolate. The lowest peripheral cells lying above the vascular plate began early to have more dense protoplasm and somewhat thickened walls. The gland-like appearance of these cells was maintained throughout the development of the tissue.[5]

Polysaccharide storage began about 10 days after pollination. It occurred first in the upper central part of the young endosperm, and spread gradually to neighboring cells. The region soon extended diagonally toward the periphery at the dorsal side of the endosperm above the embryo. By 12–15 days after pollination, a cap-shaped zone of polysaccharide-storing cells thus appeared below the base of the silk, which spread outward in all directions as more and more cells reached an advanced stage in development. There was a gradation in degree of cellular maturity, extending from this zone of cells in the cap of the endosperm which showed maximum polysaccharide storage, to those at the base of the tissue which were least advanced in development and not yet storing polysaccharides. At the same time there was a similar range in cellular development, although much shorter, extending from the zone of more mature cells in the cap outward to the periphery. At about 18 days after pollination the zone had increased in extent until it occupied the whole upper central portion of the endosperm. In the 20-day kernel one or more layers of immature cells, which were free from polysaccharides, were still present beneath the aleurone layer in the cap, but at 25 days they had usually disappeared through the initiation of polysaccharide storage, from a small area at the center of the periphery of the cap of the endosperm. By 30 days the area had increased, occupying the major portion of the upper cap. As development pro-

[5] L. F. RANDOLPH described the glandlike cells in connection with a paper (Embryogeny and endosperm formation in maize) presented before the General Section of the Botanical Society of America, New York, December, 1928. WEATHERWAX (see footnote 4) considered these cells to have a placental function with respect to the entrance of food materials into the endosperm.

ceeded it gradually spread around the sides of the cap and downward over the base of the tissue.

Always there was a transition band of cells which separated the cells showing maximum polysaccharide storage from those in which it had not begun. In this band there was a complete series of cells in the early stages of plastid development. Figs. 5 and 6 show two cells of the transition band in the lower central part of an endosperm at approximately 15 days after pollination. The first had small plastids which were collecting about the nucleus and not yet storing polysaccharide. The second cell, situated in the endosperm above the first, had larger plastids which were grouped about the nucleus, and in which polysaccharide storage had begun.

Since the transition band represented a gradation in the progress of cellular development, it was migratory and early progressed outward to the periphery of the cap, and more gradually downward into the base until the peripheral tissue was reached. Its position in a maturing kernel of waxy corn is shown in the stippled region of fig. 10 *a*. Two consecutive stages in its downward progress in maturing sweet and flint corn are shown in figs. 14 *a*, *b*, and 15 *a*, *b*. The few basal cells in a nearly mature endosperm (fig. 14 *c*) may not store any polysaccharide. No grains of carbohydrate were found in the peripheral glandlike cells bordering the vascular plate.

The position and duration of the regions of growth in the endosperm were indicated by (1) the conformation of cells, (2) the progressive enlargement of cells, (3) the degree attained by the plastids of successive cells in the synthesis of polysaccharides, and (4) the progressive maturation of cells. While meristematic activity seemed upon these bases to be everywhere present in the periphery of the tissue, it appeared to be of longer duration in the basal half of the endosperm. Final proof as to whether the continued juvenile appearance of the cells in this region is due to delayed development or to later origin rests upon the demonstration of division figures in the cells. But whichever interpretation proves to be correct, their progressive development after plastid activity began was continuous and uniform.

In this report a cell in the endosperm is designated as immature when its plastids have accomplished little or no synthesis of poly-

saccharides; it is considered mature when carbohydrate storage by the plastids appears to be complete. Growth is indicated by plastid activity in the cells, and maturation by the changes occurring at the end of the growth period, both in a cell and in the endosperm as a whole.

3. EFFECT OF PRIMARY HEREDITARY FACTORS DETERMINING ENDOSPERM TYPES UPON POLYSACCHARIDE STORAGE.—While the ontogeny of the amyliferous cells in the endosperm was found to be uniform in the different types of corn studied, the end point reached in the synthesis of polysaccharides and the visible appearance of the units elaborated were affected by the genetic complex of the kernel. To insure clarity, the concept as to the expression of the determining hereditary factors in the endosperm and the results of their segregation (summarized in the main by LAMPE and MEYERS **16**) will be briefly reviewed.

In the non-sweet types, starchy and waxy, there are moderately large simple grains of carbohydrate. These grains in the starchy type are composed of starch. In the waxy type they are composed of a closely related substance, which has been called erythrodextrin by WEATHERWAX (**33**), and waxy starch by BRINK (**3**). The grains of starch stain blue with iodine solution, those of the waxy starch stain red. In the sweet types, ordinary sweet and waxy-sweet, there are compound grains of carbohydrate and also globules of liquid dextrin which may or may not contain small grains, similar in nature to the small grains composing the larger compound ones. The grains of carbohydrate in sweet corn stain blue with iodine but in waxy-sweet corn they stain red. The liquid content of the globules stains red with iodine. Regardless of form, size, position, and reaction with iodine, all the grains of carbohydrate when dehydrated and of sufficient size are bright, and show the dark cross in polarized light which is characteristic of starch. The liquid content of the globules fails to show the dark cross, being dark throughout.

The compound grains of carbohydrate characteristic of the sweet types of corn, originally illustrated in immature kernels of Golden Bantam by MOTTIER (**22**), are shown in fig. 7. The globules also found in sweet corn, many of which have tiny grains within them, are shown in fig. 8. The storage units characteristic of the non-

sweet types (photographed earlier by JONES 12, and BRINK 3) are represented in fig. 9.

The kind of carbohydrate and the form of the storage unit produced are determined largely by the interaction of the two pairs of hereditary allelomorphs *Su su* and *Wx wx*, established by geneticists as in the main responsible for the differences in development of the endosperm in the four genetic types of corn. The presence of the dominant factor (*Su*) is responsible for the development of the large simple grains of carbohydrate. The presence of the recessive factor (*su*) and the absence of *Su* result in the development of globules and compound grains in the sweet types, regardless of whether the dominant *Wx* or the recessive *wx* is present. The allelomorphs (*Wx*, *wx*) influence the development of the grains of carbohydrate, so that when the dominant *Wx* is present starch is synthesized, and when only the recessive *wx* is present, waxy starch is synthesized.

4. EFFECT OF CELL POSITION UPON POLYSACCHARIDE STORAGE.—It is well known that the peripheral cells of the mature endosperm of all types of corn and the units of carbohydrate which they contain are always smaller than those in the central portion. In waxy corn regional variation in plastid activity, aside from the size of the unit elaborated, was also found to occur. The cells in the central region of the cap of the kernel contained grains of waxy starch, staining red with iodine solution throughout. In cells situated progressively farther away from this central region, the red-staining grains of carbohydrate had increasingly larger center-spots of blue. At the base of the endosperm, and sometimes beneath the aleurone layer, small grains entirely blue-staining were found. These little grains, as well as the center spots in the larger ones, which were shown up after fading of the mounts had occurred, lost the blue iodine stain upon heating and regained it upon cooling, just as starch does, and are considered to be of starchy character. The immature cells of the maturing waxy kernel, whose location is indicated in the stippled region of fig. 10 *a*, were elaborating the small blue-staining grains mentioned. A similar reaction was found to be characteristic of the grains of carbohydrate in the basal cells and often in other peripheral cells of the endosperm in waxy-sweet corn.

Regional variation in plastid activity was also evident in the

endosperm of sweet corn. In the sweet and waxy-sweet types the globules in the central region of the cap often contained no granules of solid carbohydrate at all. In a zone situated at some distance away in every direction, the globules usually contained small solid granules which developed within them. These granules were characteristically of starch in sweet corn, and of waxy starch in the waxy-sweet type. In cells situated still farther outward, the plastids produced globules which contained larger and larger granules, both simple and compound. The plastids in the peripheral cells of the varieties reported characteristically gave rise only to compound grains.

The change in plastid activity from the elaboration of globules to the development of grains was definite, so that a pattern was produced which was easily visible in longitudinal sections of the kernels. The peripheral zone of cells containing compound grains, and designated as the starchy region in the diagrams of fig. 14, was much more prominent at the base of the endosperm than in the cap. The last few cells to mature sometimes produced small simple grains as in non-sweet corn. In two cases grains a few times compound were found to develop in the non-sweet types.

It appears then that the time of origin of a cell and its position in the endosperm affect the end point reached in the synthesis of polysaccharides. In the presence of the dominant factors *Su* and *Wx*, together with the rest of the heredity present, the plastids synthesized starch. The substitution of the recessive *su* or *wx* resulted in incompletion of or difference in the process of synthesis, so that the liquid dextrin or the waxy starch was produced. The change in the end point reached in the synthesis of polysaccharides in the waxy and sweet types was more nearly complete in the central region of the cap, where the cells matured early in the development of the tissue. It was least evident in the peripheral cells, particularly in those at the base, where the cells matured progressively later after those in the central region of the cap had reached maturity.

5. EFFECT OF MODIFYING HEREDITARY FACTORS UPON EXPRESSION OF GENETIC FACTOR (*su*) IN ENDOSPERM OF SWEET CORN.[6]—JONES (12) segregated out by inbreeding and selection a semi-starchy type

[6] This concept was originally presented in a paper (The mode of expression of factors influencing carboyhdrate storage in the endosperm of corn) by LOIS LAMPE and MARION T. MEYERS before the Joint Genetic Sections of the American Society of Zoologists and the Botanical Society of America, Washington, December, 1924.

of sweet corn which he called pseudo-starchy. He concluded from its behavior in breeding experiments that the modified character of the kernels is not due to imperfect segregation, or to contamination of the genetic factor (*su*), but to the sorting out and rearrangement of other hereditary factors affecting its expression. JONES analyzed mature kernels of the starchy, pseudo-starchy, and sweet types macrochemically, and separated out the water-soluble carbohydrates, which would include the sugars present. The fraction from starchy corn yielded 4.45 per cent dextrose; that from pseudo-starchy 7.19 per cent; and that from sweet corn 26.15 per cent. Since the percentage of water-soluble carbohydrate in the fraction from starchy corn is approximately that obtainable as total sugar from mature kernels, the water-soluble polysaccharide appears to be absent in this type. After correction for sugars is estimated in the percentages given for the other two types, it is seen that there is less water-soluble polysaccharide in pseudo-starchy than in good quality sweet corn. The corresponding amounts of water-insoluble carbohydrates calculated as starch from a second fraction were respectively 58.73 per cent in starchy corn, 51.50 per cent in pseudo-starchy, and 29.00 per cent in sweet corn.

The idea advanced by LAMPE and MEYERS (**16**), that the water-soluble polysaccharide is furnished by the globule content of the cells in the sweet types, explains JONES' analyses. In good quality Golden Bantam sweet corn the number of globule-containing cells was large when compared with the number of cells containing only compound grains, while in the pseudo-starchy type, judging from an extensive examination of material of the JONES' strain, the proportion of cells containing globules to those containing compound grains was small. Some variation in the size of the globule-producing region also occurred in the kernels on the same ear and from plant to plant. Both storage units were absent in starchy corn where only the large simple grains were found. The extent of the core of globule-containing cells in immature kernels of Golden Bantam sweet and Jones pseudo-starchy varieties and its absence in starchy corn are shown in fig. 11. Thus in the endosperm of good quality sweet corn, the influence of the primary genetic factor (*su*) in the presence of the rest

of the heredity results in the development of compound grains and globules in about equal proportions. On the other hand, in the pseudo-starchy type the modifying hereditary factors present affect the expression of the primary genetic factor (*su*), so as to result in a

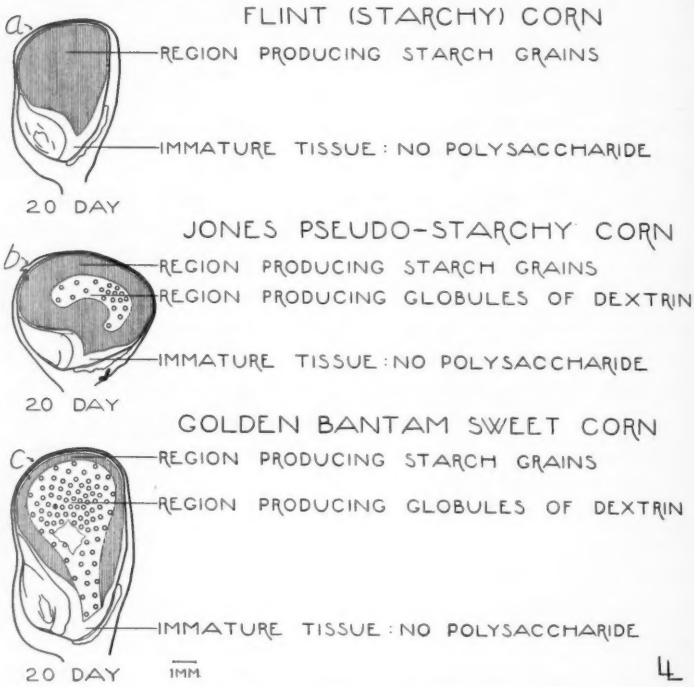

FIG. 11.—Longitudinal sections of kernels of starchy, Jones pseudostarchy, and sweet corn 20 days after pollination, showing regions of kernel whose cells give rise respectively to moderately large starch grains and globules of liquid dextrin which may or may not contain small grains; cavity often present in center of older endosperm shown in *c*.

decrease in the number of cells producing globules and an increase in the number producing only compound grains. These data indicate that other hereditary factors rearranged by segregation, and considered by JONES to be responsible for the modified character of the endosperm of the pseudo-starchy type, affect development of

the endosperm through their effect on plastid activity in the synthesis of polysaccharides.

It may be seen, however, that the factor for sweet endosperm is still operating even if no globules are produced, because of the abundant compound grains determined by it. Extreme variation in the pseudo-starchy type of sweet corn, leading to the exclusive production of the large simple grains of polysaccharide characteristic of the non-sweet types instead of the globules and compound grains, has not been found.

6. EFFECT OF REGIONAL PROGRESS OF GROWTH UPON LOCALIZATION OF CARBOHYDRATES IN DEVELOPING ENDOSPERM.

Polysaccharides.—As described in section 2, the cells in the endosperm first reaching maturity formed a zone in the cap of the endosperm which early spread outward to the periphery and more gradually downward toward the base by the addition of other cells attaining a like stage in development. Since cellular maturity was accompanied by equivalent progress in plastic activity, there was an attendant accumulation of polysaccharides in these cells. The extent of the region affected was thus at any time a measure of the amount and distribution of these substances. Figs. 10, 11, 14, and 15 show various stages in the development of the endosperm and the progress made in polysaccharide storage.

With the exception of the sugars in the embryo, the occurrence and distribution of carbohydrates in the kernel outside of the endosperm was not studied in detail. It was noted, however, that starch was not present in the body of the ovule, and that it diminished during development of the kernel in the pericarp and embryo. It was usually absent from the mature pericarp. The distribution of starch and sugars in the pericarp and that of starch in the embryo are not recorded in the illustrations.

Reducing sugars.—Reducing sugars were present during the early development of the kernel in all the types of corn, where they were found in moderate quantities in the pericarp, ovule, and young endosperm. At this time the path of translocation seemed to be in part through the succulent outer tissues. After the disappearance of the body of the ovule, and the stretching and differentiation of the cells in the upper pericarp, reducing sugars were not abundant in the ker-

nel outside of the endosperm, and appeared to enter the endosperm through the vascular plate, a disk-shaped complex of vascular tissue at the base of the pericarp.

At successive stages in the development of the endosperm, reducing sugars were confined to the immature cells where they were present in uniformly moderate amounts. The relative stage in the development of the endosperm was apparent, not only in the extent of the distribution of polysaccharides, but also in the accompanying regional distribution of reducing sugars. In older kernels the polysaccharides occurred in the more mature cells in the cap, and the reducing sugars in the immature cells at its base. As the region containing polysaccharides increased during development of the tissue, the region containing reducing sugars decreased (figs. 12 *A*, 13 *A*).

Total sugars (*sucrose by difference*).—Sucrose was not abundant in the endosperm until 10–12 days after pollination. It increased rapidly and reached a maximum throughout the tissue approximately 15 days after pollination in the several varieties studied. As the cap cells gradually matured the amount of sucrose in them decreased. Sucrose was not depleted in these cells as rapidly as the reducing sugars, however, and restriction of the region of cells containing sucrose lagged both in time and extent behind the diminishing region containing reducing sugar. During the entire growth period total sugars continued to be abundant in the basal portion of the tissue. The region indicated gradually decreased in extent and the sugar disappeared at the maturity of the kernel. Figs. 12 *B* and 13 *B* show the distribution of total sugars in the endosperm and embryo of the developing kernels of sweet and starchy corn. In each of the figures comparison may be made of the diagrams in series *A*, which show the distribution of reducing sugars in the endosperm and their absence in the embryo, with the diagrams in series *B* showing total sugars in these structures. The approximate amount and distribution of sucrose, the non-reducing sugar present, may thus be obtained. Total sugars in an older kernel of waxy corn are shown in fig. 10 *b*, where depletion of sugars was accentuated by a period of storage before the tests were made.

Amount and distribution of total sugars in different endosperm types.—While the maximum amount of total sugars reached early in

the development of the endosperm varied little in the different varieties, the amount subsequently present at any one time was specific

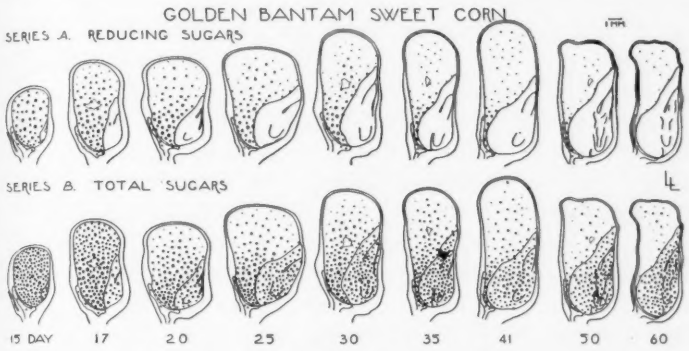

FIG. 12.—Longitudinal sections of kernels of Golden Bantam sweet corn at successive stages during growth period; amount and distribution of sugars as indicated by osazone test both before and after inversion with invertase shown by stippling: *A*, reducing sugars; *B*, total sugars; sucrose indicated by difference.

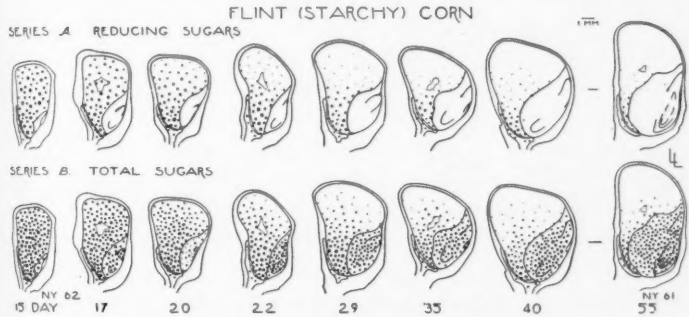

FIG. 13.—Longitudinal sections of kernels of flint corn at successive stages during growth period; amount and distribution of sugars as indicated by osazone test both before and after inversion with invertase shown by stippling: *A*, reducing sugars; *B*, total sugars; sucrose indicated by difference.

for the types of corn studied. Owing particularly to the lower amount of sucrose present, such samples of developing non-sweet corn, both starchy and waxy, and of the JONES pseudo-starchy variety, appeared regularly to contain less total sugars than com-

parable stages of good quality sweet corn, both sweet and waxy-sweet. The relative abundance of sugars in the sweet and non-sweet types is represented in figs. 12 and 13.

Differences in the types were also apparent in the amount of total sugars present in the mature endosperm. In non-sweet corn the disappearance of sugars was essentially complete at maturity. The condition is indicated in fig. 13, which shows successive stages in the development of flint corn, and in fig. 10 *b*, showing a maturing kernel of waxy corn. Sugar was present in the base of the endosperm at all stages represented, however, since no completely matured kernel is shown.

In the sweet varieties, and to a much less extent in JONES pseudo-starchy, a small amount of sugar (mostly sucrose) remained in the mature endosperm, being essentially confined to the central region where the dextrin of globule origin was localized. The small amount and distribution of total sugars in maturing kernels of Golden Bantam sweet corn are indicated in the older kernels of fig. 12 *B*. Reducing sugars which were even more restricted are shown in the maturing kernels of this variety in fig. 12 *A* and fig. 14.

Comparison of results obtained by microchemical and macrochemical methods.—The results obtained microchemically in the present study are consistent with the quantitative data obtained by CULPEPPER and MAGOON (**5**) in their periodic study of sweet and starchy varieties during the first 30 days after pollination. Both kinds of data show that there is a gradual reduction in the amount of reducing sugars, and that there is a rise and fall in the amount of sucrose. The microchemical results explain the quantitative data, however, in as much as they show (1) that there is not a general uniform decrease in reducing sugars per cell throughout the endosperm, but rather a restriction in the number of cells containing them; and (2) that there is an increase and subsequent decrease in the amount of sucrose per cell which is followed by a restriction in the number of cells in which sucrose is abundant.

The quantitative data obtained by STRAUGHN (**28**) from analyses of different parts of the mature kernel of sweet corn are also in agreement with the microchemical observations as to the amount and distribution of sugars present. Comparison of the 60-day kernel in

fig. 12 with the results of STRAUGHN, indicates (1) that sucrose and no reducing sugar occurs in the embryo, (2) that the amount of sucrose present in the endosperm is greater than that of the reducing sugars, and (3) that the amount of total sugars present in the mature endosperm is greater in the cap than in the base.

The analyses of PEARL and BARTLETT (**23**), made on mature kernels of starchy corn, agree with the microchemical results in indicating a complete disappearance of reducing sugars from the kernels during maturation. No dextrose was found by these investigators in nine determinations on six different samples, and an average of 0.12 of 1 per cent is obtainable from three determinations on two others. The same samples yielded respectively 1.83 and 1.71 average percentages of sucrose. The microchemical method showed that the sucrose reported in the macrochemical analysis was in the embryo. Maturing kernels are shown in figs. 10 *b*, 13, and 15, although no completely matured kernel is illustrated.

7. REGIONAL CHANGES IN SAP CONTENT OF MATURING ENDOSPERM. —In the foregoing sections of the paper, it was shown: (1) that the ontogeny of an amyliferous cell in the endosperm resulted in abundant polysaccharide storage, and that maturing of the cell was accompanied by a relative reduction in the amount of cell sap; (2) that regional progress in cellular development occurred first in the cap, and spread gradually to the base of the tissue during later stages of development; (3) that a transition band of cells showing all degrees of maturity occurred between the regions of maturing and immature tissue; (4) that this transition band migrated downward to the base of the older endosperm as cells successively entered upon the period of polysaccharide storage; (5) that polysaccharide storage in the cells of this band marked the beginning of the decrease in the relative amount of sap present in the cells.

As a result of the further decrease of sap content during the drying down of the kernel, a second transition band appeared in the endosperm above the one just mentioned. This new zone was narrow, and separated the cells in the cap of the kernel, which contained little or no sap and were completely mature, from those below it, which still contained abundant sap and were immature. Since final maturation and rapid loss of the remaining cell sap appeared to occur in this

zone of cells, it was called the zone of maturation. The line of demarcation was definite and associated with the external appearance of the kernel known as glazing.

The maturation zone was migratory, as was the transition band of less mature cells just mentioned. Its lowering from the center of the periphery of the cap to the base of the tissue is called in this paper the fall of the zone of maturation. The lowering of this zone was more rapid when drying down first began, because the cells in the cap were everywhere nearly mature and free from sap. Later its lowering was less rapid, because the cells at the base of the endosperm approached maturity and complete polysaccharide storage successively, at the same time lessening the reserve of immature sap-filled cells.

The presence and fall of the zone of maturation were found to be characteristic of the endosperm of all the types and varieties of corn studied. In these experiments the fall of this zone in waxy corn had progressed one-third of the way down the endosperm at 35 days after pollination, and at 40 days, in the flint, Golden Bantam sweet, and Jones pseudo-starchy varieties. It had not begun to fall at 35 days in Burr-Leaming dent and waxy-sweet corn.

Successive positions of the zone of maturation in the maturing endosperm of Golden Bantam sweet corn are shown in fig. 14 *a*, *b*. It was no longer apparent in the endosperm shown in *c*. Quantitative data as to the amount of dry matter and the moisture content of the kernels are also included in fig. 14. From these it is evident that there was a decrease in the percentage of moisture and a corresponding increase in the percentage of dry matter in progressively older kernels.

When the endosperm was separated from the embryo and divided at the line of cutting indicated (fig. 14), the moisture content in all three samples was found to be greater in the base of the endosperm than in the cap. When the percentages of moisture in the caps and in the bases of the successive samples are compared, it is apparent that the loss of moisture in the base was greater during the maturation period than the loss in the more nearly mature cap. When the kernel was practically mature (fig. 14 *c*) the percentage of moisture was nearly uniform throughout.

The position of the zone of maturation in maturing kernels of the

non-sweet varieties is shown in figs. 10 *a* and 15. When kernels of flint corn were prepared as were those of Golden Bantam just described, the amount of moisture was again found to be greater in the base than in the cap at the two stages studied. The loss of moisture during the early part of the maturation period here studied was also

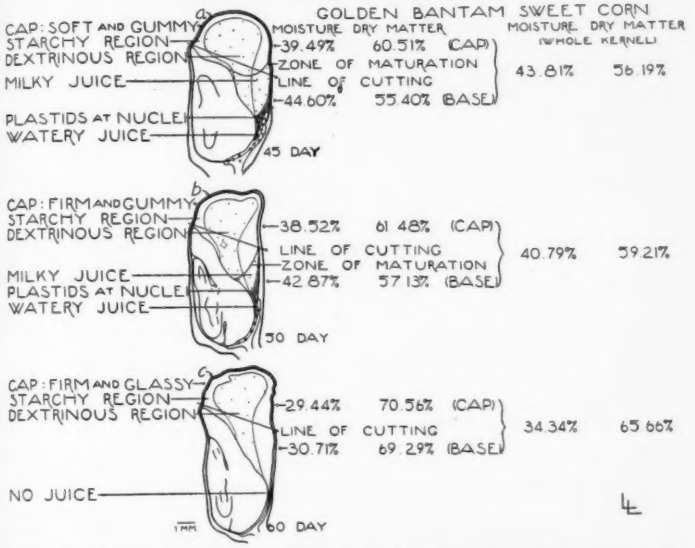

FIG. 14.—Longitudinal sections of kernels of Golden Bantam sweet corn made during later stages of development, showing (1) regional progress in cellular development and maturation; (2) position of gross internal regions of kernel and zone of maturation; (3) moisture and dry matter content of cap and base of endosperm when separated at line of cutting indicated; and (4) moisture and dry matter content of whole kernel. Amount and distribution of reducing sugars shown by stippling.

greater in the base, where the cells were progressively arriving at maximum polysaccharide storage (fig. 15). Greater differences were found in this type than in Golden Bantam sweet corn, for which three factors were primarily responsible: (1) the samples were from two successive plantings; (2) there was a greater difference in the number of days between pollination and collection for the samples used; and (3) the chemical nature of the polysaccharides synthesized was specific for the two types of corn. The changes in moisture con-

tent during maturation of the kernels show the same regional development of the endosperm as is indicated by the regional progress in the maturing of the cells, and by the consequent localization of sugars and polysaccharides.

The characteristics of the gross internal regions of the endosperm which are present during the maturation period determine the gross external appearance of the kernel subsequent to premature or normal drying down of the kernel.

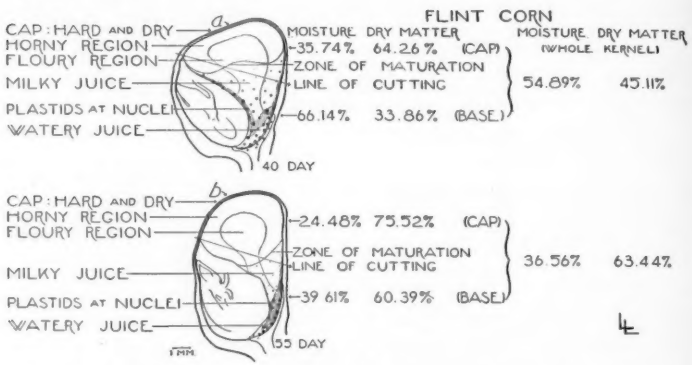

FIG. 15.—Longitudinal sections of kernels of flint corn made during later stages in development, showing (1) regional progress in cellular development and maturation; (2) position of gross internal regions of kernel and zone of maturation; (3) moisture and dry matter content of cap and base of endosperm when separated at line of cutting indicated; and (4) moisture and dry matter content of whole kernel. Amount and distribution of reducing sugars shown by stippling.

Kernels of flint corn 40 days after pollination had succulent tissue at the base of the endosperm, and hard dryish tissue in the cap where the cells were well packed with starch. The base of the endosperm was found to shrink upon drying in proportion to the size of the region affected, and to the degree of succulence present. The cap, however, remained plump and rounded because the cells were well filled with starch, and little moisture was present which could be lost. In the practical judging of corn, kernels shrunken at the base have long been penalized. MOORE (21) pointed out that shrunken kernels are indicative of immaturity, and that the shrinkage results in spaces at the cob, between the bases of the kernels. LYON and MONTGOMERY

(17) state that such spaces indicate immaturity, poor feeding value, and a decreased percentage of grain. On the other hand, kernels of flint corn in which the storage of starch had been practically completed in the basal cells of the endosperm, and which lacked succulent tissue here, did not materially change their shape upon drying. A high starch content and low percentage of moisture throughout the kernel resulted in maintenance of the original form. MOORE (21) recorded that in well matured corn no noticeable spaces are found at the cob between the bases of the kernels, and that they fit tightly together at the base as well as at the cap. Effects similar to those described for flint corn were apparent also in the kernels of the other types studied, where changes in the cap due to degree of packing or to kind of carbohydrate present complicated in certain ones the results obtained. The gross internal regions of the maturing endosperm of waxy, sweet, and flint corn are indicated respectively in figs. 10 *a*, 14, and 15.

8. PERCENTAGES OF MOISTURE AND DRY MATTER IN DEVELOPING KERNEL.—CULPEPPER and MAGOON (5) have already shown that a progressive decrease in moisture content and a like increase in dry matter occur during the earlier stages in the development of the corn kernel. They also found an increase in polysaccharide content which parallelled closely the increase in amount of dry matter. The percentages of moisture calculated for the samples are shown in the curves of fig. 16. Microscopical study of the kernels indicated that decrease in the moisture content of the kernel as a whole began at initiation of polysaccharide storage in the cells of the endosperm, and continued regularly until the drying down of the kernel occurred. Corresponding increase in the percentage of dry matter paralleled somewhat closely the successive maturing of the cells, as indicated by their progress in polysaccharide storage. Thus the percentages of moisture and dry matter in the kernels constitute a fair index to the maturity of the endosperm. The percentage of moisture in the kernel, however, could be an absolute index only provided the embryo and pericarp showed the same changes in moisture content.

The data from the samples of the different types which were of the same age in days after pollination are not entirely comparable, mainly because of the differences in the times of pollination. The

samples from Golden Bantam sweet, flint, and Jones pseudo-starchy varieties, which were pollinated earlier, had a lower average percentage of moisture; and those of Burr-Leaming dent and waxy-sweet, which were pollinated later, had a higher average percentage

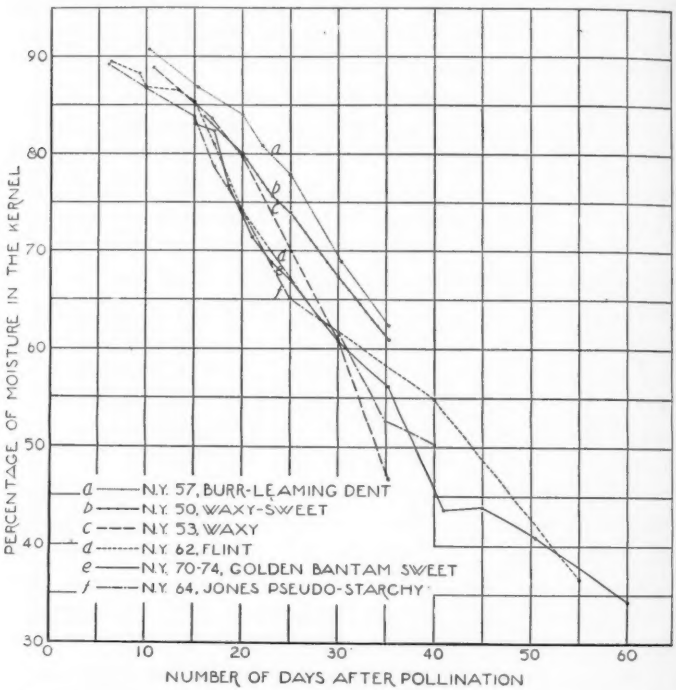

FIG. 16.—Curves showing moisture content of kernels at successive stages of development in the six varieties of corn studied.

of moisture. Hence the curves of fig. 16 fall into two groups. The curve of the waxy type, however, passed from the later to the earlier group. This variety also appeared to mature more rapidly than the two late-pollinated ones.

Discussion

Development of the endosperm in the types of maize studied was characterized by morphological and physiological gradients which

are undoubtedly associated with polarity and symmetry in growth. Regional gradients in cellular development and consequent distribution of materials were evident in the growing endosperm. Such gradients may be assembled into two groups. The members of the one were shorter, and extended inward from the periphery of the endosperm at the cap of the kernel where the meristematic aspect of cells first disappeared. Those of the other group, which continued with decreasing prominence throughout the period of development, extended upward toward the cap from the base of the endosperm where the cells, owing either to arrested development or to more recent origin, retained a juvenile appearance for a much longer time.

Since the ontogeny of a cell ended in abundant polysaccharide storage, there were gradients in these materials which extended from an ever widening region in the upper part of the endosperm outward toward the periphery and downward into the base. After maturing of the cells in the cap, gradients in the amount of materials present also ceased to exist there. Progressively shorter gradients in reducing sugars, sucrose, and moisture content extended from the base upward toward the cap. They virtually disappeared at kernel maturity.

There were also gradients in the character of the polysaccharides synthesized in the waxy and sweet types which extended outward in all directions from the central region in the cap of the endosperm. It was suggested in section 4 that the time of appearance of a cell, and consequently its position with reference to the periphery of the endosperm, affected its plastid activity and the end point in polysaccharide synthesis. Development of every cell appeared thus to be influenced by the presence of those which arose before it, and perhaps to some extent by those which came afterward, and correlations were indicated in the development of the different parts.

The economic importance of the investigation lies primarily in the recognition of the regional maturing of the endosperm in relation to yield and quality of grain at harvest. The continued cellular activity in the base of the tissue explains to a great extent why THATCHER (**29**) and SHELTON (**26**) found an increase in yield proportional to lateness in time of harvesting, and also why kernels properly developed and matured are well filled at the base and of maximum dry

weight, as required in the judging of corn. Recognition of the regional maturing of the endosperm has an important bearing also upon the time of collection of sweet corn for best edible quality.

Summary

Progressive development of the endosperm in the four genetic types of maize (starchy, waxy, sweet, and waxy-sweet) was studied by microchemical and microscopical methods.

1. ONTOGENY OF AMYLIFEROUS CELL.

a. Appearance of an amyliferous cell was followed by enlargement of the whole cell, its vacuole, and nucleus. The size attained by the cells of an older endosperm was greater in the central region of the tissue than at the periphery.

b. The plastids developed from granules, the protoplastids, which were present in the cytoplasm. In the more advanced cells, 7–12 days after pollination, the plastids were grouped about the nucleus. At about 10 days, polysaccharide storage began in the more advanced cells, and differentiation in the plastids of the four genetic types was evident.

c. The plastid population of a single cell appeared to develop uniformly and concurrently. The plastids were imbedded in the cytoplasmic network which finally filled the cell.

d. The relative amount of vacuolar sap in a cell decreased during its development, and the vacuole ceased to be observable.

e. The grains of polysaccharide elaborated by the plastids in waxy corn increased in size up to 30 days after pollination. At this time those in the cells of the region which later became horny were angular, and fitted closely together; those in the region which later became floury were loosely packed, and spherical. No further significant change in the cell and its contents occurred until the drying down of the kernel.

2. ONTOGENY OF ENDOSPERM.

a. Peripheral meristematic activity appeared to be everywhere present early in the development of endosperm tissue, and to be of longer duration in the base than in the cap.

b. Polysaccharide storage began first in the upper central part of the endosperm tissue.

c. Regional progress in the development of cells early resulted in a cap-shaped zone of cells, advanced in polysaccharide storage, which was situated in the upper part of the endosperm below the base of the silk. This zone of cells increased in size, spreading outward to the periphery of the cap, and much more gradually downward into the base of the endosperm by the addition of other cells attaining a like stage in development.

d. There was a transition band of cells in which polysaccharide storage was just beginning, which separated the cells showing maximum polysaccharide storage from those in which it had not begun. The band was migratory, and early progressed outward to the periphery in the cap and more gradually downward into the base of the tissue.

3. EFFECT OF PRIMARY HEREDITARY FACTORS DETERMINING ENDOSPERM TYPES UPON POLYSACCHARIDE STORAGE.

a. The end point reached in polysaccharide synthesis in the endosperm and the appearance of the units elaborated are determined in the main by the hereditary allelomorphs *Su, su* and *Wx, wx*.

b. In the endosperm of non-sweet corn, presence of the dominant factor (*Su*) results in the development of rather large simple grains of carbohydrate.

c. In the endosperm of sweet corn, presence of the recessive factor (*su*) and absence of *Su* results in development of compound grains of carbohydrate and globules of liquid dextrin. The latter may or may not contain small grains.

d. The allelomorphic pair (*Wx, wx*) affects development of the grains of carbohydrate. When *Wx* is present, starch is synthesized as in the endosperm of starchy and ordinary sweet corn. When *wx* is present and *Wx* absent, waxy starch is synthesized as in the endosperm of waxy and waxy-sweet corn.

4. EFFECT OF POSITION OF CELL UPON POLYSACCHARIDE STORAGE.

a. In waxy corn the grains of waxy starch situated progressively farther away from the central region of the cap had increasingly larger centers of starchy character. Grains entirely of starch were often produced in the peripheral cells, particularly in those at the base of the tissue. The latter were also found located similarly in waxy-sweet corn.

b. In good quality sweet types the cells in the upper central region contained only globules of liquid dextrin. At some distance outward in every direction, the plastids produced globules which contained progressively larger granules of solid carbohydrate. The plastids in the peripheral cells usually produced only compound grains. This change was definite and easily visible in longitudinal sections of the kernels.

5. EFFECT OF MODIFYING HEREDITARY FACTORS UPON EXPRESSION OF PRIMARY GENETIC FACTOR (*su*) IN ENDOSPERM OF SWEET CORN.

In the endosperm of the pseudo-starchy type the modifying hereditary factors present affected the expression of the primary genetic factor (*su*), resulting in a decrease in the number of cells producing globules, and an increase in the number producing only compound grains. The central core of the globule-producing cells of the Jones pseudo-starchy variety was therefore greatly reduced when compared with that in good quality sweet corn.

6. EFFECT OF REGIONAL PROGRESS OF GROWTH UPON LOCALIZATION OF CARBOHYDRATES IN DEVELOPING ENDOSPERM.

a. Polysaccharides accumulated first in the more advanced cells in the cap, and the region extended progressively outward and downward as the maturing of cells proceeded.

b. Reducing sugars decreased in the cap as the cells approached maturity. They were present in uniformly moderate amounts, in successively fewer immature cells in the basal half of the endosperm.

c. The sucrose content of the endosperm in the several varieties rose gradually to its maximum amount and distribution in the tissue at about 15 days after pollination, and then decreased. The sucrose content of the cells was not lowered as rapidly as that of reducing sugars, however, and restriction of the region containing sucrose lagged both in time and extent behind the diminishing region containing reducing sugars.

d. The amount of total sugar in the endosperm subsequent to about 15 days after pollination was greater in good quality sweet corn than in the Jones pseudo-starchy and non-sweet types.

e. In non-sweet corn disappearance of sugars from the endosperm was essentially complete at maturity. In the sweet types, and to a

less extent in the Jones pseudo-starchy variety, a small amount of sugar (mostly sucrose) remained in the mature endosperm, being practically confined to the central region where the dextrin of globule origin was localized.

f. Data obtained by the quantitative chemical analysis of corn kernels are supplemented by the results arrived at microchemically, since localization of materials in the kernel is thereby made available.

7. REGIONAL CHANGES IN SAP CONTENT OF MATURING ENDOSPERM.

a. At the drying down of the kernel the cells successively and rapidly lost their remaining sap and entered upon the period of maturation. The narrow zone of cells in which this change was occurring was called the zone of maturation. It separated the cells in the cap of the older endosperm which had matured and were practically free of sap from the immature sap-containing cells in the base.

b. The zone of maturation migrated downward as development proceeded. Its lowering from the center of the periphery to the base of the tissue was termed the fall of the zone of maturation. This fall usually began in the kernel 35–40 days after pollination. At the beginning the fall was more rapid because the cells were nearly mature and free from sap. Later it was less rapid because the cells at the base of the endosperm approached maturity and complete polysaccharide storage successively.

8. PERCENTAGES OF MOISTURE AND DRY MATTER IN DEVELOPING KERNEL.

a. The percentage of moisture steadily decreased as development of the kernel proceeded.

b. Increase in dry matter paralleled closely the progress made in the synthesis of polysaccharides as the cells of the endosperm successively attained complete polysaccharide storage.

Gradients in the development of cells and in the attendant localization and distribution of materials, such as the carbohydrate and moisture content, suggest that polarity and symmetry underlie the harmonious development of the endosperm. Correlation between the position of the cell and its plastid activity is indicated by the regional

variation in the nature of the polysaccharides synthesized in the waxy and sweet types.

The economic importance of the investigation lies in the recognition of the regional maturing of the endosperm in relation to time of selection of sweet corn for best edible quality, and in relation to maximum yield of grain at harvest time.

The writer wishes to acknowledge her indebtedness to Dr. H. C. SAMPSON of the Ohio State University, and to Dr. S. H. ECKERSON of the Boyce Thompson Institute under whose direction the investigation was made; to Dr. WILLIAM CROCKER for the privilege of continuing the problem at the Institute; and to Mr. MARION T. MEYERS for selecting and furnishing the seed used and for cooperation and help given. Appreciated advice and criticism have also been obtained from Professor JOHN H. SCHAFFNER.

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EXPLANATION OF PLATE III

Amyliferous cells and polysaccharide bodies resulting from plastid activity in endosperm of corn. Figs. 1–6 inclusive are from free-hand sections of fresh tissue mounted in a mixture of 5 per cent sugar solution and weak iodine potassium iodide solution; figs. 7–9 are from smears using tissue in the cap of the endosperm and very dilute iodine solution. Magnification in figs. 2–4 inclusive is slightly less than that in the other figures (×0.87).

FIG. 1.—Golden Bantam sweet corn approximately 5 days after pollination; protoplastids scattered throughout cytoplasm.

FIG. 2.—Waxy corn 9 days after pollination; plastids collecting about nucleus.

FIG. 3.—Waxy corn 11 days after pollination; plastids mostly grouped about nucleus, larger, and beginning to store polysaccharides.

FIG. 4.—Waxy corn 17 days after pollination; plastids scattered about within cytoplasm mainly at one side of cell.

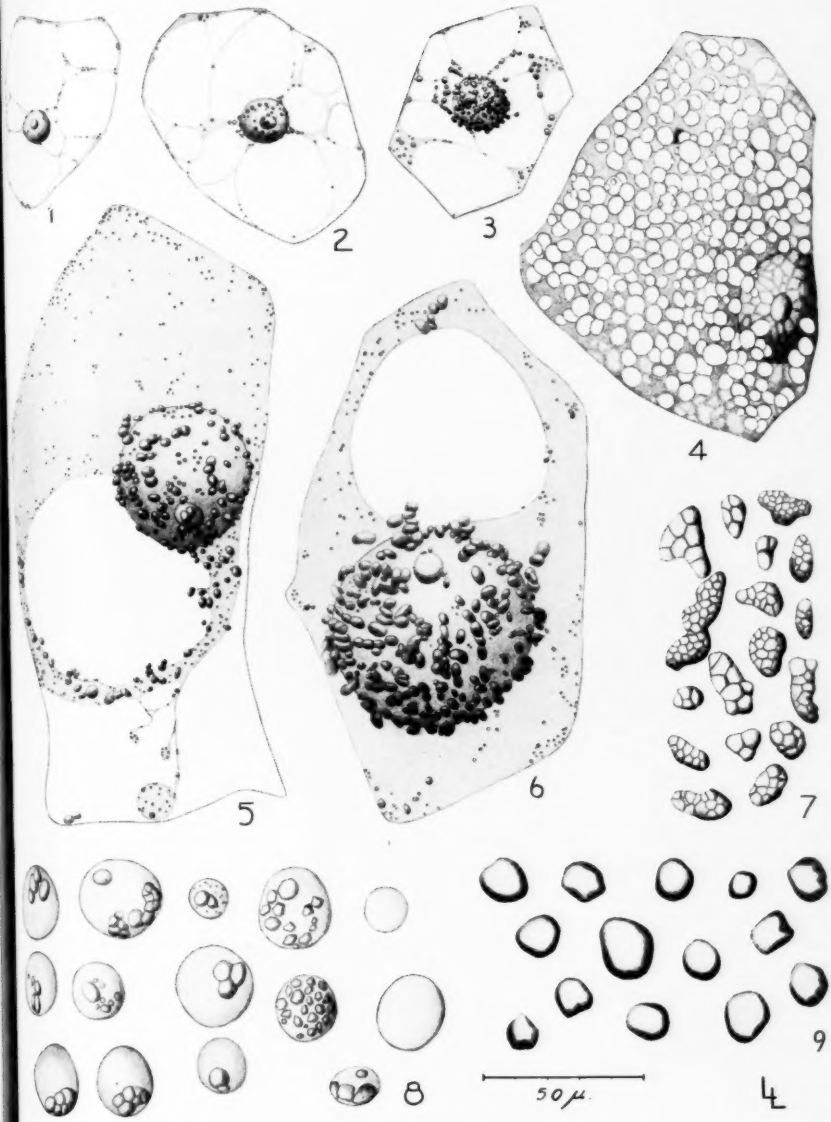
FIG. 5.—Golden Bantam sweet corn approximately 15 days after pollination; cell in basal half of endosperm showing plastids collecting about nucleus; some plasmolysis of cell apparent.

FIG. 6.—Same; cell above that shown in fig. 5; plastids showing starch synthesis grouped about nucleus; structure of plastids not shown.

FIG. 7.—Black Mexican sweet corn 50 days after pollination; compound grains of starch from cells in endosperm above tip of embryo.

FIG. 8.—Same; globules of liquid dextrin, many of which contain starch grains; from cells at center of cap of endosperm.

FIG. 9.—Burr-Leaming dent corn 50 days after pollination; simple grains of starch from horny and floury regions in central part of endosperm cap.



LAMPE on MAIZE

DEVELOPMENT OF DIONAEA MUSCIPULA

II. GERMINATION OF SEED AND DEVELOPMENT OF SEEDLING TO MATURITY[1]

CORNELIA MARSCHALL SMITH

(WITH THIRTY-SIX FIGURES)

Preparation of soil

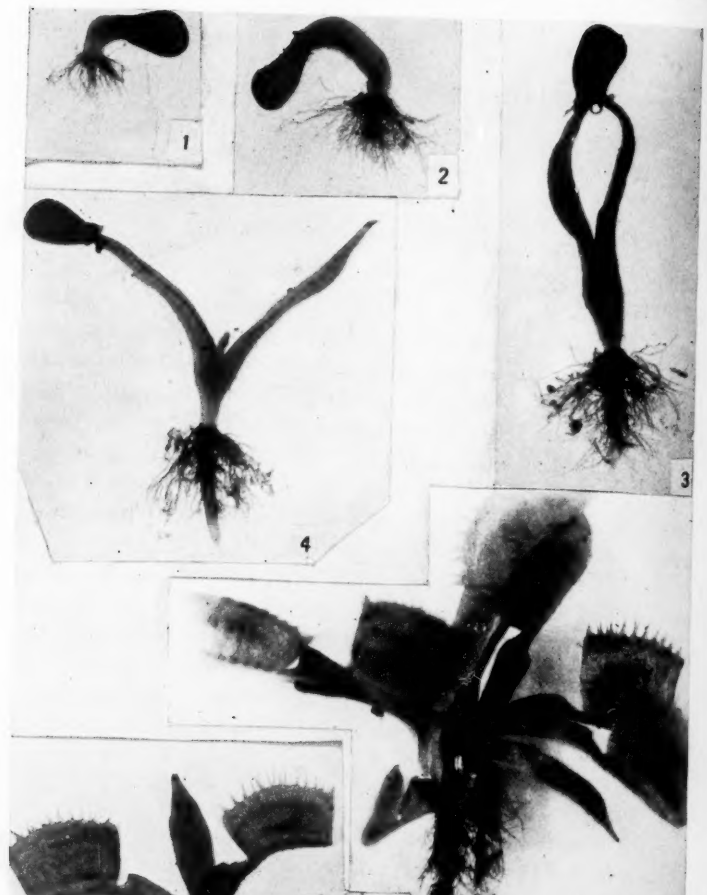
The seeds of *Dionaea* are difficult to germinate. Of the various conditions under which the writer attempted to grow them, the following proved to be the most successful. The bottom of a porous clay flower pot, 5 inches in height and 6 inches in diameter, was covered with pieces of broken pottery, on top of which was placed a layer of sand and then a mixture, 2 inches in depth, of sand and humus collected from a bog where *Drosera* and *Sarracenia* grew. The seeds were scattered over the surface of the soil, which was sprinkled with water in order to bury them slightly. The flower pot was covered with a plate of glass, taken into a greenhouse, and placed in a concrete tank containing just enough running water to keep the soil within continuously moist. The temperature of the greenhouse was maintained at 25°–35° C. Under these conditions some of the seeds germinated in 10 days, some in 2 weeks, others not until the lapse of 3 weeks, and a number of them had not germinated when the experiment ended.

Mature seed

A longitudinal section of a mature latent seed (fig. 7) shows the broad face of one of the cotyledons, the stem growing point, and the hypocotyl of the embryo. The embryo is surrounded at its micropylar end by a single-layered cellular structure, which fits over it like a cap and extends to the copious endosperm. These structures are invested by a thin inner and a rather thick, black, outer seed coat.

In the writer's previous paper (**25**), in which the origin and development of the endosperm were traced, it was shown that the endosperm contains a bountiful supply of starch. According to later

[1] Botanical contribution from The Johns Hopkins University, no. 110.



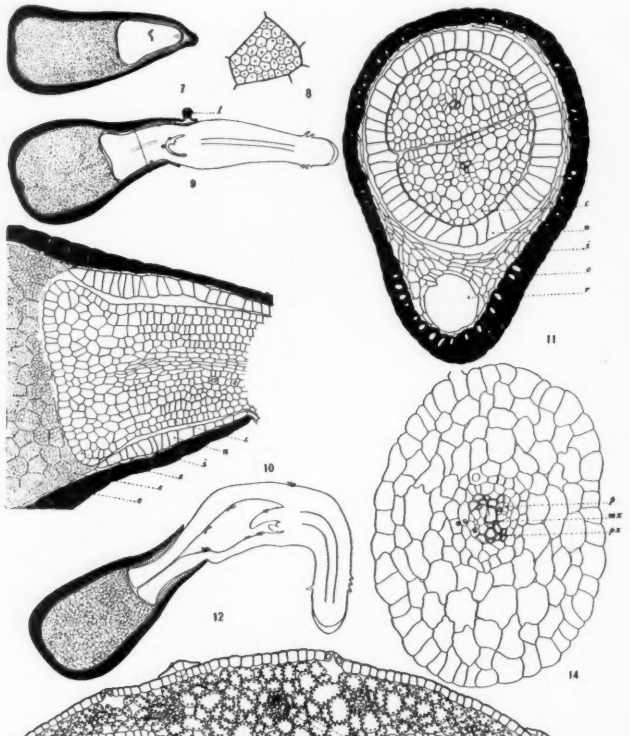
FIGS. 1–6.—*Dionaea muscipula:* stages of development in germination; ×10

study, the starch grains are usually pentagonal or hexagonal in cross-section (fig. 8), and vary from 5 to 7 μ in diameter. The persistent nucellar cells cap the micropylar end of the embryo sac, and are not encroached upon and disintegrated by the growth and expansion of the embryo and endosperm, as is the remainder of the nucellar tissue. A longitudinal section of an ovule containing an immature embryo shows the exact position of these cells, which lie above the embryo and fit closely into the micropyle (**25,** fig. 63). A similar section of a mature seed (fig. 7) shows these cells surrounding the embryo to the tip of the cotyledons, where they seem to connect with the endosperm. Although the nucellar cells and endosperm appear to merge into a single structure, they differ in that the former does not contain starch grains whereas the latter does, and in that they arise independently. Figs. 10 and 11, longitudinal and transverse sections through the narrow end of a germinated seed, show the large-celled nucellar tissue surrounding the two closely appressed cotyledons to the place where their tip ends are in contact with the endosperm.

The situation in *Dionaea* admits comparison with that of *Peperomia hispidula*, described by JOHNSON (**13**). In *Dionaea* the food is stored in the endosperm, whereas in *Peperomia* it is stored in the perisperm; in *Dionaea* the embryo is surrounded, with the exception of the tips of the cotyledons, by the persistent nucellar tissue, while in *Peperomia* it is completely invested by the endosperm. JOHNSON suggests that the endosperm of *Peperomia* serves as a medium of transfer of food between the perisperm and embryo, and that it serves as a stopper protecting the germinating seedling from loss of dissolved food, from desiccation, and from the entrance of fungi. It does not seem probable that the persistent nucellar cells in *Dionaea* function as mediums of transfer, since they do not surround the tip ends of the cotyledons. They appear, however, to form an adequate seal against the escape of dissolved food from the endosperm, and most likely prevent the entrance of fungi, as does the endosperm in *Peperomia*.

Seed germination

The first sign of germination of the seed visible from the exterior is the pushing off of the lid by the hypocotyl, at the lower end of which the primary root is developing. This lid, derived solely from



FIGS. 7–14.—Sections of seed and stages in its germination: fig. 7, longitudinal section of mature, latent seed; fig. 8, detail of single endosperm cell containing starch grains; fig. 9, longitudinal section of entire seed and seedling in early stage of germination; fig. 10, detail from fig. 9 showing end of cotyledon and tissues surrounding it; fig. 11, cross-section of seed and seedling at level indicated by dotted line in fig. 9; fig. 12, longitudinal section of seed and seedling at approximately stage shown in fig. 2; fig. 13, cross-section of cotyledon; fig. 14, cross-section of primary root (*c*, cotyledon; *e*, endosperm; *i*, inner seed coat; *l*, seed lid; *mx*, metaxylem; *n*, nucellar tissue; *o*, outer seed coat; *p*, phloem; *px*, protoxylem; *v*, cavity of vascular bundle; *z*, papillate epidermal cell).

the inner integument, often remains attached along one side of the seed (figs. 2, 4, 9). The short hypocotyl and the primary root grow directly downward, and the latter becomes attached to the soil by numerous dark brown root hairs (fig. 1). A longitudinal section of the seed made at this time shows that, in making their exit, the hypocotyl and primary root not only push off the seed lid, but rupture the persistent nucellar layer (fig. 9). The tips of the cotyledons possess slightly elongated, papillate epidermal cells which serve as haustoria, transporting food from the endosperm, with which they are in contact, to the median vascular bundle of their narrow, elongated, basal portion (figs. 9–11). Before the cotyledons emerge, the initial cells of the stellate hairs appear on them, and at approximately the same time they appear on the first secondary leaf developing at the stem growing point (fig. 9).

As the seedling continues its development, the basal ends of the cotyledons, as a result of intercalary growth, elongate and emerge from the seed (fig. 2), become green, and straighten up (fig. 3). The spatulate tips, which originally composed the greater part of the cotyledons, do not increase in size; they remain inclosed and continue to absorb food. A longitudinal section of the seed and seedling (fig. 12) shows the endosperm in direct contact with the tips of the cotyledons, the elongated basal portions of which are studded here and there with stellate hairs, the embryonic leaves arising at the stem growing point, the arched hypocotyl, and finally the primary root protected by the root cap.

One of the cotyledons extricates itself completely from the seed, and the two then diverge to an angle of about 90°, young leaves becoming visible between them (fig. 4). The seed remains attached to one cotyledon even after several secondary leaves have formed (fig. 5); that is, until food absorption is completed. When the seed finally drops off, the spatulate tips of both cotyledons have shriveled and turned brown. By this time the basal part of each cotyledon has reached its full size, varying from 4 to 6 mm. in length, and from 0.1 to 1.2 mm. in width. Stellate hairs and stomata appear on both their upper and lower surfaces, and a transverse section (fig. 13) shows that the epidermis is covered with a thin layer of cutin, and that

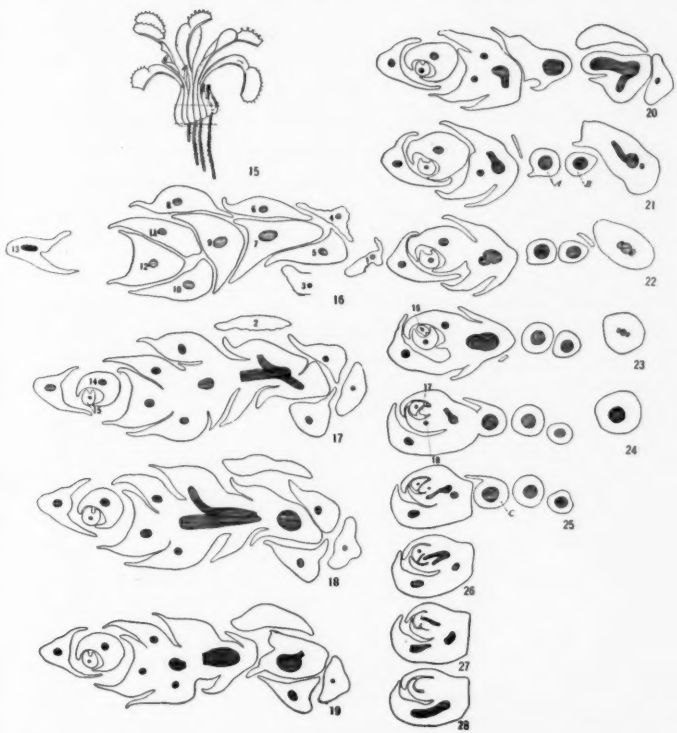
each cotyledon contains one principal vein and two smaller veins on each side, surrounded by chlorophyll-bearing parenchyma cells. The arrangement of the vascular tissues of the veins is similar to that ordinarily found in the vein of a secondary leaf of this species.

If the vascular bundles which supply the cotyledons are followed downward, it is found that as they leave the hypocotyl they are inverted, there being no transition region in the hypocotyl. Fig. 9 indicates the position, and figs. 11 and 13 show the endarch collateral arrangement of the vascular elements of the cotyledons. There is no transition region in the hypocotyl, since throughout its entire length, and that of the primary root, the vascular bundles do not alter their course, and orientation of the tissues of the bundles is similar. Since the only notable difference between the hypocotyl and the primary root is the addition of a few xylem and phloem parenchyma cells in the stele of the hypocotyl, a description of the tissues contained in one of these structures will suffice. In a transverse section of the primary root (fig .14) the following parts are to be distinguished: the epidermis, composed of one layer of cells from which root hairs may arise; the cortex, consisting of three or more layers of parenchyma cells; the endodermis; the pericycle; and the diarch xylem rays alternating with the phloem. No secondary or branch roots are formed. The growing end of the primary root is protected by a root cap, which arises from a common initial zone of the root apex, and is similar to that of the adventive root shown in fig. 33.

The greatest number of root hairs develop on the primary root slightly below the soil line, and are remarkably straight and long (figs. 1–5). They range from 0.4 to 1.9 mm. in length, and are able to resist rough handling; for when soil particles are removed from around them, even with the aid of a camel's hair brush, they escape uninjured. Inasmuch as the chemical composition of their walls seems to be identical with that of the root hairs of the mature plant, the composition of these will be discussed together later.

As the seedling continues to develop leaves, the stem apex moves from between the cotyledons to a position on a level with and external to the proximal end of the cotyledons, and by continued elongation in the same direction, a horizontal rhizome, the apex of which is protected by young leaves, is gradually formed (fig. 6). Adventitious

roots originating from the lower surface of the rhizome, soon begin to appear. Usually six to eight leaves and two or three adventive roots are formed when the seedling, consisting of cotyledons, hypo-



FIGS. 15–28.—Fig. 15, entire seedling, showing, between dotted lines, region from which sections for figs. 16–28 were made; figs. 16–28, series of selected horizontal sections of rhizome of seedling shown, in outline, with vascular content shaded; starting with section shown in fig. 16, sections from which the 12 succeeding figures were drawn are 190, 280, 360, 460, 600, 690, 880, 1080, 1120, 1150, and 1220 μ respectively from it; leaves numbered 1 to 18 and roots lettered *A* to *C* in order of appearance.

cotyl, and primary root, begins to shrivel, turn brown, die, and is sloughed off at the proximal end of the rhizome of the now independent plant.

Mature plant

HOLM (11) has called attention to the fact that the first leaves formed, although greatly reduced in size, are identical in structure with the mature leaf, which for decades has held the interest of scientists. Since the anatomy of the blade of the leaf has been investigated and described by OUDEMANS (18), DARWIN (2), KURTZ (15), DE CANDOLLE (1), FRAUSTADT (5), GOEBEL (6), HABERLANDT (9), GUTTENBERG (8), and others, there is no necessity to discuss it here. The basal part of the leaves, rhizome, and roots will however be described. To study the relationship of these parts, 10 μ serial longitudinal sections were cut horizontally (parallel to the soil surface) through a 9-months old plant, beginning at the bases of the leaves just above the rhizome. The space between the horizontal lines in fig. 15 indicates the region of the plant from which the sections were made, and figs. 16–28 show in outline the contents of certain selected sections.

The rhizome usually grows in a directly horizontal plane, but at times the apical end is buried more deeply in the ground than the proximal end. This is particularly true of young plants. The leaves arise in close spiral succession from the apex of the rhizome (figs. 16–28). As they mature they extend first laterally and then upward, the blade and expanded part of the petiole of each unfolding in a circinate manner above ground, leaving only the base of the petiole underground. The petioles of the older leaves, whose bases overlap, inclose and protect the tender embryonic leaves. It is this close set, spiral arrangement which gives the mature plant its characteristic rosette-like appearance. Fig. 29 shows that the base, as has previously been pointed out by various investigators, also serves as a storage organ and contains an abundant supply of starch. The starch grains (fig. 30) are somewhat larger than those found in the endosperm, they are ovoid in outline, and vary from 5 to 15 μ in length. Each petiole may contain one vascular bundle, or in older plants one principal bundle and two smaller, lateral ones on each side. The endarch arrangement of the elements of the bundle (fig. 29) is that typical of the petiole of a leaf. The bundle is completely surrounded and even penetrated by parenchyma cells, which appear to contain a pectic substance.

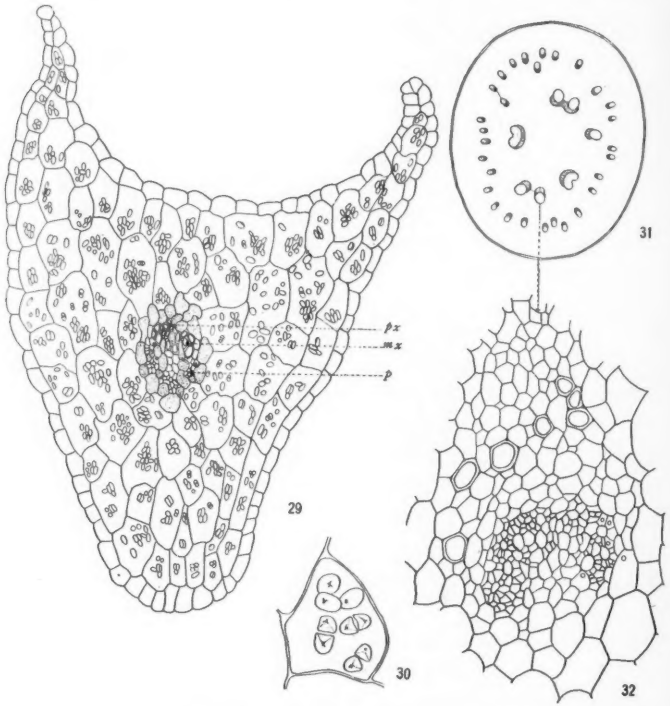
Following the vascular bundle of any leaf to its source (figs. 16–28), it may be observed that it originates from the upper surface of the centrally located vascular tissue of the rhizome. The bases of the petioles of two adjacent leaves not only overlap but are attached, forming a storage and protective covering around the vascular tissue, which is inclosed by a layer of cells, apparently containing a pectic material, similar to that found in the leaf. The rhizome appears to consist of a composite of leaf bases with several vascular bundles coursing through them.

The flower stalk contains a dissected ectophloic siphonostele (figs. 31, 32). This indicates that a similar arrangement of the vascular tissue is entirely possible and even expected in the rhizome.

The roots originate in an almost median line from the ventral side of the vascular tissue, near the apical end of the rhizome. As each develops, it makes its way either through the region where two adjoining leaf bases overlap and are attached to each other, or, less frequently, through the enlarged middle portion of a single leaf base, carrying along a shield-shaped piece of the leaf base (fig. 34), which may be seen surrounding the proximal end of the mature root. Like the rest of the leaf base, this shield-shaped piece has stellate hairs scattered over its surface. Usually three to six functional roots, varying 2–8 cm. in length and 0.5–0.9 mm. in diameter, project downward from the rhizome.

The stele of the root of the young seedling may be diarch, as is the stele of the primary root, or tetrarch (fig. 35), whereas that of the roots of the older plants is most often octarch (fig. 36). This increase in the number of strands is possibly due, as fig. 34 seems to show, to a division of the two primary strands. A root possessing octarch bundles was described and figured by FRAUSTADT (**5**). Figs. 34–36 show that the roots of *Dionaea* contain a normal series of tissues: epidermis, cortex, endodermis, and stele which consists of pericycle and xylem and phloem strands. The tracheids in each vascular strand, contrary to FRAUSTADT'S description, are few in number and irregularly distributed, being separated from those of the adjacent strand by xylem parenchyma. A dark staining material, similar to that in the cells surrounding the vascular tissue of the rhizome and leaf, is found in some of the cells of the cortex, endodermis, and

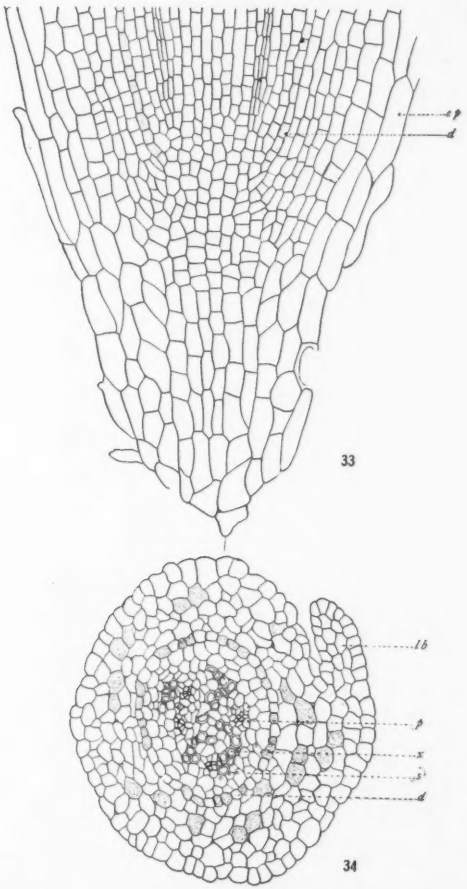
pericycle, and frequently in some of those investing the phloem. In older roots the epidermis and a part of the cortex may be sloughed off, and, when this happens, this dark-staining material appears to collect in the cells of the pericycle on the side where the cortex is



FIGS. 29–32.—Fig. 29, detail of leaf petiole no. 12 in fig. 16; fig. 30, detail from fig. 29 of single starch-containing parenchyma cell; fig. 31, cross-section of flower scape showing arrangement of vascular bundles; fig. 32, detail of single bundle from flower scape (*mx*, metaxylem; *p*, phloem; *px*, protoxylem).

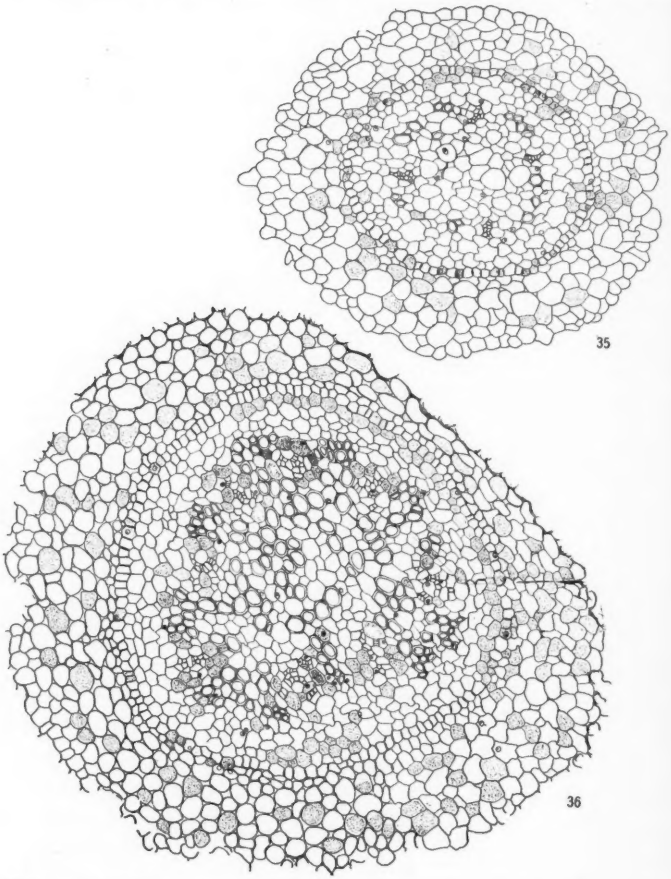
sloughed off (fig. 36). The growing end of the root is protected by a root cap (fig. 33), which originates like the root cap of the primary root from a common initial zone of the promeristem. A few millimeters behind the growing point, root hairs begin to form which, like those of the primary root already described, soon become thick-

walled and turn brown. They may persist throughout the life of the root, and may even be found on the old roots which have ceased to function and are being sloughed off the proximal end of the rhizome.



FIGS. 33, 34.—Fig. 33, longitudinal section of root tip showing root cap arising from common initial zone; fig. 34, detail of root *A* in fig. 21, showing 4 groups of tracheids separated by xylem parenchyma, two distinct phloem strands, and third phloem strand in process of separation (*d*, endodermis; *ep*, epidermis; *lb*, portion of leaf base; *p*, phloem; *s*, pericycle; *x*, xylem).

In an effort to ascertain their composition, the cell walls of the root hairs were tested microchemically as follows: for cellulose, with



FIGS. 35, 36.—Fig. 35, cross-section of root slightly older than one in preceding figure, showing fewer tracheids, more parenchyma, and 4 separate phloem strands; fig. 36, cross-section of octarch root from old plant, showing epidermis and part of cortex sloughed off.

concentrated sulphuric acid, fresh cuprammonia, iodine and sulphuric acid, and with chloriodide of zinc both before and after treatment

with nitric acid and potassium chlorate; for lignin, with phloroglucin and hydrochloric acid, and with aniline sulphate and sulphuric acid both before and after bleaching with sodium hypochlorite; for callose, with resorcin blue; for pectose, with ruthenium red, hydrochloric acid, and potassium hydroxide; for calcium pectate, with ammonium oxalate; for suberin and cutin, with alkanin, chlorophyll, cyanin, and Sudan III, before and after treatment with both potassium hypochlorite and potassium hydroxide. Although the root hairs of *Dionaea* responded to none of these tests, which include those normally used for determining the composition of the cell walls of root hairs, they were finally dissolved after 2 hours' gentle boiling in either a 50 per cent solution of chromic acid or in Schulze's maceration fluid, or after remaining in potassium hypochlorite for 3 full days. Since chromic acid is a recognized test for cutin, and since the root hairs of *Dionaea* responded to this test, they probably contain this substance, although the other tests failed to reveal its presence. The root hairs of *Dionaea*, therefore, appear to be vestigial structures which have ceased to function.

Systematic position of Dionaea muscipula

There is some diversity of opinion among recent taxonomists as to the position of the Droseraceae. ENGLER and GILG (**4**) and RENDLE (**21**) include the three families Sarraceniaceae, Nepenthaceae, and Droseraceae in the order Sarraceniales. HUTCHINSON (**12**) includes the Sarraceniaceae and Droseraceae in the Sarraceniales, but places the Nepenthaceae, because of their woody structure, in the order Aristolochiales. DIELS (**3**), monographer of the Droseraceae for *Das Pflanzenreich*, in discussing the relationship of this family, attaches particular importance to the hypogynous insertion of the parts of the flower, and the truly parietal placentation of the ovules in *Drosera*, and therefore places the Droseraceae under the order Parietales, nearest akin to the Violaceae.[2] The same scheme is followed by WETTSTEIN (**26**), who, however, places the Sarraceniaceae and Nepenthaceae in a separate order, the Polycarpicae. While these taxonomists agree in regarding the four genera, *Drosophyllum*, *Dio-*

[2] DIELS gives an account of the different positions in which the Droseraceae had been placed by taxonomists up to 1906.

naea, *Aldrovanda*, and *Drosera*, as composing the family Droseraceae, SMALL (**24**) places *Dionaea* in a separate family, the Dionaeaceae. It is of interest to see what light the features of the development of *Dionaea muscipula* recorded in the present paper, as well as in the earlier paper (**25**), throw on its position in the plant kingdom.

In *Dionaea*, as in *Drosophyllum*, 10–20 (most often 15) stamens are formed, whereas in both *Drosera* and *Aldrovanda* only five are developed. The nuclear phenomena of the microsporocytes of *Dionaea* are essentially like those of *Drosera rotundifolia* and *D. longifolia* described by ROSENBERG (**22**). Furthermore, judging from ROSENBERG'S figures, the chromosomes of *Drosera* appear to be similar in shape and form to those of *Dionaea*. He gives 10 and 20 as the haploid number of chromosomes respectively in *D. rotundifolia* and *D. longifolia*, whereas 15 is the haploid number in *Dionaea*. The pollen grains of *Dionaea* hang together in tetrads, a characteristic of the entire family. In structure they appear to be similar to the pollen grains of *Drosera squamosa* as described by DIELS (**3**).

One of the most distinctive parts of the flower of the Droseraceae is the pistil. In *Dionaea* and *Drosophyllum* it is composed of 5 carpels, and contains numerous seeds on a basal placenta. Whether the ovary of *Drosophyllum* is "paracarpous," and bears its ovules without the aid of the vegetative apex of the flower, as described by GOEBEL (**7**) for *Dionaea*, or whether the vegetative apex shares in the formation of the ovary, has not been reported, nor has the writer had an opportunity to investigate this situation. Judging from DIELS' figures, however, one would be inclined to conclude that they develop as in *Dionaea*. In *Aldrovanda* and in *Drosera*, the ovary is syncarpous, in the former consisting of 5 carpels, in the latter 2–5 (most often 3). In both genera the ovules develop on marginal parietal placentas.

While all four genera have anatropous ovules, there are striking similarities as well as differences in the development of the ovules of *Dionaea* and of *Drosera*. In *Dionaea* the hypodermal archesporium divides to form two or sometimes three layers of parietal cells above the embryo sac, whereas in *Drosera* (PACE **19**) parietal cells are commonly not formed, although sometimes a single layer develops between the epidermis and the mother cell. Furthermore, the enlargement of the outer layer of nucellar cells of *Dionaea* resembles that

of *Drosera rotundifolia* described by Miss PACE, but the fate of the cells in *Dionaea* (**25**) is different from that suggested by her for *Drosera*. Moreover, a comparison of the mature embryo sac of *Dionaea* (**25**) with that of *Drosera* (**19**) shows the two to be similar. *Dionaea* develops a seed lid not markedly different from that in *D. rotundifolia* described by NETOLITZKY (**17**).

Dionaea is the only one of the Droseraceae in which the persistent nucellar tissue has been observed and described. The slides of *Drosera rotundifolia* made by PETERS (**25**), when compared with slides of *Dionaea*, show that the endosperm of the two passes through practically the same stages of karyokinetic differentiation and development. The embryo of *Dionaea*, as is true of that of the other members of the family, is small, and lies at the base of the seed in direct contact with the copious endosperm.

A comparative study of germination of the seeds of the four genera of the Droseraceae shows the following gradation in the development of the primary root and cotyledons. (1) Neither *Aldrovanda*, as described by KORZCHINSKY (**14**), nor *Drosera*, as described by HEINRICHER (**10**) and later by DIELS (**3**), develops a vigorous primary root; instead, each forms a rudimentary root which functions for a brief time only. (2) *Dionaea*, according to HOLM (**11**), develops a primary root, which, as the present investigation shows, ceases to function and is sloughed off with the cotyledons after leaves and adventitious roots have formed and the plant has become independent. (3) *Drosophyllum*, according to PENZIG (**20**), develops a primary root which persists. On the other hand, the four genera vary with respect to the function and development of the cotyledons: in *Aldrovanda*, according to KORZCHINSKY (**14**), and in *Drosophyllum*, according to PENZIG (**20**), the cotyledons remain within the seed and absorb food from the endosperm; whereas in *Drosera*, according to HEINRICHER (**10**) and DIELS (**3**), and in *Dionaea*, only the tips of the cotyledons do this. The remaining and greater part of the cotyledons emerge and carry on the process of photosynthesis. Germination of the seed of *Dionaea* closely resembles that of *Sarracenia* and *Darlingtonia* described by MACFARLANE (**16**).

Since there are numerous characters upon which the four genera agree exactly, since there is a smaller number upon which at least two genera agree, and since there are only a few upon which all the

genera differ, it seems logical to concur with those who place *Dionaea* among the Droseraceae, and divide the family into the four genera *Drosophyllum*, *Aldrovanda*, *Dionaea*, and *Drosera*. Furthermore, a comparative study of the morphology of the flowers of the Droseraceae and the Sarraceniaceae and Nepenthaceae, although showing that these differ as to number of stamens, number of carpels, and placentation of ovules, definitely indicates their affinity. Among the Sarraceniaceae the stamens vary from 10 to ∞; in *Sarracenia* there are 70–80, which, according to SHREVE (**23**), originate as ten groups of primordia; in *Darlingtonia*, according to MACFARLANE (**16**), 15 is the typical number formed. Among the Droseraceae, on the other hand, the stamens range from 5 to 20; in *Dionaea* and in *Drosophyllum* there are 10–20 (most often 15), which, in *Dionaea* and probably in *Drosophyllum*, originate as in *Sarracenia* as ten groups of primordia; in *Drosera* and in *Aldrovanda* 5 are regularly produced. The pistil shows the following variations: in *Aldrovanda* it consists of 5 and in *Drosera* 2–5 (most often 3) united carpels, which bear their ovules on marginal, parietal placentas; in *Drosophyllum* and in *Dionaea* it is made up of 5 carpels, which develop ovules on their united upraised bases; in *Sarracenia* and *Darlingtonia* it has 5, and in *Heliamphora* 3 united carpels, which grow inward and develop their ovules on axillary placentas. Apparently, therefore, the three families should be placed in the order Sarraceniales.

Summary

1. Germination of the seed and development of the seedling of *Dionaea* proceed as follows: the hypocotyl and the primary root elongate, rupture the nucellar cap, and push aside the seed lid; the cotyledons partially emerge, turn green, and straighten up, carrying the seed with them; one cotyledon releases itself completely and the other remains attached by its tip end, the two then diverging and young leaves appearing between them. The stem apex, which gives rise to leaves in spiral succession, gradually moves horizontally from between the cotyledons, forming a subterranean rhizome from the lower surface of which adventive roots arise; the cotyledons, hypocotyl, and primary root finally cease to function and are sloughed off the basal end of the rhizome of the now independent plant.

2. The main aspects of the anatomy of the seedling are as follows: the hypocotyl and the primary root are similar in structure, both having epidermis, cortex, endodermis, pericycle, and a diarch stele; there is no transition region in the hypocotyl, the vascular bundles being inverted where they enter the cotyledons; the cotyledons, with stomata and stellate hairs present on both upper and lower surfaces, have a median collateral endarch vascular bundle from which two or more lateral branches extend.

3. The rhizome appears to consist of an agglomeration of overlapping undiverged leaf bases with several vascular bundles coursing through them.

4. The stele of the root of a young plant is usually tetrarch, whereas the stele of the root of a mature plant is most often octarch.

5. The root hairs, which begin to form a few millimeters behind the growing point, soon turn brown and become thick-walled, often persisting throughout the life of the root. Of the microchemical tests used to determine the composition of their cell walls, the chromic acid test for cutin alone gave positive results. They appear, therefore, to be vestigial structures.

6. The evidence gained from a comparative study of the development of *Dionaea* and the plants closely related to it indicates that *Dionaea* should be placed in the family Droseraceae and in the order Sarraceniales.

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ASCENT OF SAP IN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 417

FANNY SMITH, R. B. DUSTMAN, AND CHAS. A. SHULL

(WITH ONE FIGURE)

The ascent of sap in plants, particularly tall plants, was long a puzzle to botanists and physicists. Finally a satisfactory explanation of the lifting of water in even the tallest trees was given by the work of DIXON (4), ASKENASY (1), RENNER (7), BODE (2), and others (6, 9) during the last 35 years. The explanation is given in terms of such forces as the evaporational power of water, the surface tensional and imbibitional forces of the cell colloids of the leaf, the osmotic behavior of water in the presence of semipermeable or differentially permeable membranes (especially protoplasmic membranes), and the cohesional properties of the tracheal sap. All of these forces have been found adequate for the work. The average force of water evaporation into a complete water vacuum has been estimated at 1350 atmospheres; the imbibitional forces developed by drying organic matter range from zero to about 1000 atmospheres, depending on the degree of drying; osmotic forces up to 205 atmospheres (10) have been found in the living cells of leaves; and the cohesion of water is probably not less than 300 to 350 atmospheres. Since the total force required for lifting water to a height of several hundred feet is estimated at approximately 20 atmospheres, these forces can all operate at modest fractions of the possible development of power. In view of these facts, the cohesion theory of sap ascent has been rather generally accepted as the most reasonable explanation of sap rise phenomena.

One recent author, however, holds to another theory. BOSE (3) believes that the water is pumped upward through the inner cortical regions of the stem by pulsating cells in which hydraulic waves of contraction and relaxation succeed one another up the stems of the plant. This idea of BOSE's is probably based upon a complete misconception of the nature of certain electrical phenomena which he

claims to have observed, and which he has interpreted in terms of pulsations similar to that of a beating heart.

It is interesting to note that both BOSE and DIXON propose a vitalistic cause of sap ascent. BOSE's views can be laid aside without discussion, since his location of sap ascent in the cortex is contrary to the experience of all others who have worked upon this problem. Even if others were able to demonstrate the electrical disturbances BOSE claims to have observed, there is no reason for conceding the interpretation which he has placed upon them.

A different theory of vital action is proposed by DIXON. He assumes that the chief source of energy available and responsible for the rise of the tracheary columns of water in woody stems is the energy of respiration. He ascribes the removal of water from the leaf cells to an active secretion, which takes place irrespective of any evaporation which occurs into the leaf interspaces. DIXON admits that evaporation may exceed secretion, and in such exceptional cases might exert an evaporational pull upon the water columns; but the ordinary and usual water ascent is brought about by the active secretion, after which the water is evaporated, without the evaporation having any part in the development of the forces of water rise. This idea of an active secretion of water by the leaf is concurred in by BOSE, who describes the active secretion of water by the leaves of *Nauclea*. BOSE covered the leaves of *Nauclea* with vaseline, and claims that in spite of this covering the leaves kept on taking in water at the cut end of the petioles, and excreting it as liquid water under the coating of vaseline, since there was no opportunity for evaporation under these conditions.

As evidence for his rather unusual theory of active secretion of water by leaves, DIXON describes and figures experiments with branches cut under water and mounted in an inverted bell jar, in such a manner that the upper part of the branch was completely submerged in water. These operations were all carried out in such a way that the cut surfaces of the stem were never exposed to the atmosphere, in order that the water columns of the stem might not be broken, and that there might not be any clogging of tracheae with air bubbles.

The lower end of the cut stem projected through the cork which

closed the bell jar below, and was allowed to dip into a solution of eosin. Since the leaves were all submerged, there could be no evaporation of water from the leaf surfaces. In the work to be reported later the same precautions were taken to prevent entry of air into the cut stems.

Although the transpiration was stopped in DIXON's experiments, he found a continuous rise of eosin through the stem; and he used this intake of eosin at the cut end of the stem as crucial evidence of the continuous excretion of liquid from the leaves into the surrounding liquid which bathed the shoot. This rise of eosin in the branch occurs against the hydrostatic pressure upon the plant from the depth of the water in the bell jar, and DIXON ascribes to respiration the generation of the energy used to cause this excretion against the head of pressure in the bell jar.

Some years ago one of the writers (**8**) commented upon these observations by DIXON, but without going into a detailed criticism. It seemed obvious that saturation deficit in the tissues of the stems used was not sufficiently taken into consideration. Normal translocation was afterwards associated with saturation deficit effects by DIXON (**5**) in his work with potatoes, in which he described a downward translocation of sap in the tracheae, and proposed that the xylem could transfer water upward, and organic foods downward, at the same time. The present paper indicates that the rise of eosin solution observed by DIXON in his bell jar experiments may be explained solely on the grounds of a saturation deficit existing in the leaf and stem tissues at the time the branches were cut. Such a deficit must always exist in growing plants, and unless precautions are taken to relieve it, it will manifest itself in the results of such experiments. Although submerged in water, the internal water deficit continues to determine the intake of water until the deficit is satisfied. This will occur through the paths of least resistance, which in the case of a submerged plant is through the cut ends of the tracheae in the eosin bottle. Tests with various plants showed that one could easily repeat DIXON's experiences if the stems were cut under water and set up at once in the bell jar. But DIXON seems not to have made adequate provision for overcoming this saturation deficit in the leaves and branches used in his experiments. Our pre-

liminary experiments, run in the late autumn of 1922, were designed to take care of this important factor in the rise of eosin. Later the work was extended at intervals to a number of other kinds of plants during the summers of 1923 and 1926, and to include studies of transpiration with BOSE's bubbler. The latest studies involved the use of winter twigs during the latter part of the winter of 1930.

Materials and methods

In the first experiments (DUSTMAN) several cottonwood branches, fuchsias, and a shoot of *Grevillea robusta* were used. All shoots were 20–30 cm. in length, the cottonwood stems being cut in mid-December, and having large well developed winter buds. This species was chosen because of the large buds and consequent abundance of respiring material. The fuchsias and *Grevillea* plants were potted specimens from the University greenhouses, and were chosen for their large leaf areas and relatively rigid stems. The diameter of the cottonwood stems was approximately 0.75 cm., and of the others about 0.5 cm. All were cut under water, and kept continuously under water until the conclusion of the experiment. The fuchsia and *Grevillea* stems were mounted in inverted bell jars, and the cottonwood stems in inverted 2-liter bottles which were completely filled with water.

In order to overcome, so far as possible, any saturation deficit in the leaves and stems, the potted plants were placed with their roots in pans of free water beneath closed bell jars for 24 hours before the branches were cut. The condensation of free water on the sides of the bell jars indicated that this had largely been accomplished. The stems were then cut under water, mounted in the inverted bell jars, and, to insure saturation, allowed to stand completely submerged for a time before the eosin was added. The cottonwood twigs were not placed under bell jars previously, but were permitted to stand submerged in the bottles for 24 hours before being tested with eosin. Later results showed that this is not a long enough period to secure complete satisfaction of the saturation deficit, in the case of dormant twigs.

That the cottonwood branches contained a considerable volume of gas, and that this gas was under some pressure, was shown by the fact that when cut the stems emitted a continuous stream of tiny bubbles for a short time. This release of gases was again apparent

when the stems were fitted into the bottles. Two factors may explain the emission of gas: rise in temperature when first brought in and hydrostatic pressure when finally set up for the experiment. The hydrostatic pressure was in part due to the set up, for the bottles were completely filled with water, and the rubber stoppers through which the stems passed were fitted tightly into the bottle necks. In the most recent experiments with winter twigs, they were used in inverted bell jars so that the eosin intake would not have to take place against the compressibility of water and the elastic give of the rubber cork. The results of the later work are in harmony with those of the preliminary cottonwood twig experiments.

The later work (SMITH), during the summers of 1923 and 1926, involved several types of experiment: (1) transpiration of branches with and without saturation deficit into saturated atmosphere; (2) secretion experiments with submerged plants some of which had been relieved of water deficit and others not; and (3) secretion experiments with leaves, using BOSE's bubbler method with vaseline-coated leaves.

The studies of sap rise in a saturated atmosphere were made with a variety of species. Young and vigorous branches of the plants listed in table I were used. They were cut under water, and the cut ends kept there for 20 minutes in the dark room. Then they were transferred to a bell jar in which the atmosphere was kept as nearly saturated as possible, and still kept standing in water for an hour to give opportunity for overcoming the water deficit of leaf and stem tissues. At the end of an hour the water around the ends of the cut stems was replaced with eosin solution, and a beaker of water at 100° C. was placed within the bell jar, which was now closed and returned to the dark room. The rise of eosin was measured at the end of one hour. Similar experiments were performed with branches which had not been relieved of their saturation deficit.

For the secretion experiments the plants listed in table II were used. These were cut under water and arranged in the bell jar just as in the preliminary tests. The cut ends of the stems were kept covered with water for 2 hours, the shoot being submerged, at which time the water was replaced by eosin. After an hour the ascent of eosin in the stems was measured.

The experiments with BOSE's bubbler were performed with

healthy leaves of the plants listed in table III. The leaves were severed from the plant under water, and left for 30 minutes with the petioles in water. The leaf petiole was then passed through one of the holes of a 2-hole stopper, and sealed in air-tight with wax. During these operations the cut end of the petiole was never allowed to get out of the water. The bubbler tube was inserted tightly through the second hole of the stopper, after which the stopper was inserted into the water reservoir with the petiole projecting into the water, and sealed with an air-tight seal.

Typewriter oil or clove oil was used as the indicator valve for the bubbler. The rate of transpiration is determined by the number of bubbles of air which pass the oil valve in a definite time interval. After the set-up had stood for one hour, to give the leaf time to dry and become adjusted to the conditions of the experiment, and to determine the normal rate of transpiration while uncoated, the leaf was carefully and thoroughly coated with vaseline. The readings were extended over a period of 30–60 or more minutes after the leaves had been coated.

The latest studies with winter twigs (SHULL) were made with stems cut under water, and kept there for various periods of time after being arranged in the bell jars. Care was taken to avoid great changes of temperature by having at hand considerable volumes of water which had come to room temperature. The factor most likely to give trouble in such experiments is the internal atmosphere of the tracheal system. Slow solution of the gases in water on standing is likely to occur, with some rise of eosin. Any lowering of temperature will cause contraction of gases, and the registration of some rise of eosin; for even if later on the gas is expanded to its original volume, the eosin has stained the xylem as far up as the contraction of gases drew it.

Twigs of *Ulmus americana*, *Ptelea trifoliata*, *Rhus canadensis*, *Cornus stolonifera* (?), and *Crataegus* sp. were used. After a 12-hour period with the cut ends in eosin, the twigs were split, and observations made of the rise of eosin into the tracheal system.

Results

The preliminary experiments indicated that the rise of eosin was probably controlled by saturation deficits in the tissues. The cotton-

wood branches, containing air, are very difficult to saturate, and during slow solution of the internal gases some eosin would enter the stems, owing to this cause and to slight fluctuations of temperature. One twig, submerged 24 hours and allowed to stand in eosin solution for 19 hours, showed only 2 cm. rise. Two others, submerged for 48 hours and then arranged in eosin, showed in the first of the two a rise of 5.5 cm. in 2 days, and in the second a rise of 5.0 cm. in 5 days. One would expect a much greater rise in 5 days than in 2 days, if the rise were due to continuous excretion of water. The three controls,

TABLE I

RISE OF SAP OF PLANTS IN SATURATED AIR

PLANTS USED	LENGTH OF BRANCH (CM.)	TREATMENT			RISE OF EOSIN (MM.)
		Time in H_2O in dark (min.)	Time in H_2O in bell jar (hours)	Time in eosin (hours)	
Quercus macrocarpa.....	27	20	1	1	2
Ribes odoratum.....	30	15	1	1	3
Philadelphus grandiflorus.	25	15	1	1	2
Ulmus americana.....	30	20	1	1	2
Ailanthus glandulosus....	47	20	1	1	3
Ginkgo biloba.....	31	20	1	1	4
Robinia pseudacacia.....	34	20	1	1	3
Catalpa speciosa.....	36	20	1	1	2
Tilia americana.....	30	20	1	1	3
Acer saccharinum.....	35	20	1	1	3
Platanus occidentalis....	43	20	1	1	2

allowed to stand in air instead of being submerged, and then in eosin for the same length of time (19 hours, 2 days, 5 days), showed 13 cm., 16.5 cm., and complete traversing of the stem respectively.

The fuchsias, prepared as previously described, showed only 1 mm. rise in 19 hours, and only 1 mm. in 48 hours respectively, for the two stems tested. And the *Grevillea robusta*, kept in the eosin solution for 5 days after the saturation deficit was relieved, showed a rise of only 1 mm. This same stem was then freshly cut and exposed to air for 5 hours, standing in eosin. The rise of the eosin was 30 cm. during that period. The small rises noted in the fuchsias and *Grevillea* are easily accounted for by diffusion alone.

The experiments designed to test the rise of sap in plants kept in a saturated atmosphere are shown in table I.

The branches used varied in length from 25 to 47 cm., and the rise

of eosin in the cut stems was in no case over 4 mm. in an hour. In the majority of cases it was only 2–3 mm. One must believe that this small rise is due mainly to diffusion of eosin into the stem, and not to active secretion of water from the leaf.

In numerous other experiments the branches were set up in a saturated chamber with the ends dipping into water for 3 hours. At this time the water was replaced with eosin for 12–24 hours, and the rise noted. These branches were then removed and allowed to wilt for 1.5–2 hours, after which they were returned to the eosin, and the tops exposed in a saturated atmosphere under a bell jar. Ten species were employed. Not a single shoot showed significant rise of eosin during the first period under the bell jar; but after wilting, all of them showed rise on account of the saturation deficit induced during wilting. The largest rise occurred in *Ginkgo* and *Philadelphus*, which showed 15 cm. rise during the 24 hours; the smallest was in *Cornus florida*, which showed a rise of 1.5 cm. The average rise of duplicate experiments with all ten species was 7.36 cm. These results are in strict agreement with those of table III, and indicate the significance of the saturation deficit in connection with sap movement.

Fifteen species of plants were used in the experiments with submerged shoots. One branch was always set up in air to show the rise of fluids in the branch during normal transpiration, where evaporation accompanies sap rise. The results are presented in table II. As is seen from the data, when the plants were submerged, and given one or two hours to imbibe water before applying eosin to the cut stems, there was in no case any significant rise of sap.

The greatest rise, 5 mm., was found in *Ribes odoratum*, and in most cases the eosin had penetrated only 2 or 3 mm., which might be expected as the result of diffusional entry. We were unable to obtain any evidence confirmatory of the secretory theory of sap ascent, for whenever the saturation deficit was really satisfied before the eosin was applied, there was no significant sap movement in the stems.

The last two columns of table II present the results obtained when control stems are set up in air. The contrast with the rise in stems relieved of saturation deficit is evident. In a number of instances, after standing surrounded by water with no appreciable rise of

eosin, the water was removed from the bell jars and the plants exposed to air with the cut ends still dipping into eosin. Every shoot showed considerable rise, and in some branches the dye penetrated to the tips of the shoots.

Branches were also placed in the bell jars, immersed in water, and given the eosin at once, without giving an opportunity to satisfy the water deficit existing in the twigs. There was always a rise of the

TABLE II

ASCENT OF SAP IN SUBMERGED SHOOTS

PLANTS USED	LENGTH OF BRANCH (CM.)	TREATMENT			RISE OF EOSIN (MM.)	RISE IN CONTROL	
		Time left in H_2O (hours)	Temp. of H_2O (° C).	Time in eosin (hours)		(cm.)	(min.)
Quercus macrocarpa	38	2	23	1	3	35	28
Ribes odoratum	29	1	21	1	5	33	39
Philadelphus coronarius	34	1	20	1	3	22	26
Chrysanthemum							
Wm. Turner	31	2	21	2	2	30	30
Black Hawk	27	2	21	2	2	28	27
Chrysolaria	40	2	20	1	3	9.5	30
Ulmus americana	49	1	20	1	2	38	29
Ailanthus glandulosus	43	1	21	1	2	41	30
Ginkgo biloba	32	1	20	1	3	29	45
Robinia pseudacacia	42	1	20	1	2	29	30
Catalpa speciosa	48	1	20	1	3	30	30
Lycium vulgare	28	1	21	1	2	28	25
Philadelphus grandiflorus	35	2	20	2	4	32	30
Tilia americana	34	1	20	1	2	15	35
Ptelea trifoliata	49	1	20	1	3	16	28

eosin, in harmony with DIXON'S results with experiments set up in the same manner.

Finally we present the results of repetitions of BOSE'S work with the bubbler. Active leaves were selected for the work. *Hydrangea arborescens* leaves gave a reading of eleven bubbles in 61 minutes. After coating both sides of the leaf with boiled and cooled vaseline, one bubble was noted at the end of 28 minutes, and no more during the next hour. No water was found under the vaseline. The temperature was 24° C. during the test. The general results with leaves of ten species of plants are given in table III.

In 70 per cent of the cases no bubbles were produced after the vaseline was applied. In 30 per cent one bubble was noted after

considerable lapse of time. In not a single instance was any water found under the vaseline coating, even at the end of 24 hours. In the case of the most active leaf, *Vitis labrusca*, bubbling ceased immediately with the covering of the leaf with vaseline, and no sign of water secretion beneath the vaseline could be observed.

The results recorded in table III refute the claim of BOSE that transpiration is a secretory process. Unfortunately it was not possible to use the leaf of *Nauclea* for this experiment, as the plant is not native to this country and could not be found among exotic collec-

TABLE III

RESULTS WITH BUBBLER METHOD WITHOUT AND WITH VASELINE COATING

PLANTS USED	LENGTH OF OBSERVATION WITHOUT VASELINE (MINUTES)	NO. OF BUBBLES	NO. OF BUBBLES AFTER COATING WITH VASELINE	WATER UNDER VASELINE	TEMPERATURE (° C.)
Hydrangea arborescens	61.0	11	1 after 28 min.	None	24
Begonia sp.	70.5	14	None	"	24
Ptelea trifoliata	67.3	8	1 after 27 min.	"	25
Cornus florida	73.0	11	1 after 11 min.	"	29
Catalpa speciosa	51.6	5	None within 1 hour	"	31
Tilia americana	58.8	18	" " " "	"	32
Philadelphus grandiflorus	57.5	10	" " " "	"	32
Vitis labrusca	26.0	22	" " " "	"	28
Ampelopsis quinquefolia	64.4	6	" " " "	"	28
Viola palmata	53.0	7	" " " "	"	29

tions in local conservatories. Certainly none of the species examined behaved in the manner described for *Nauclea*. Even if BOSE's observations of the action of *Nauclea* leaves were found to be correct, it cannot represent a general behavior among plants.

The most recent tests, made with twigs in winter condition during the latter part of the winter of 1930, were in harmony with the earlier results with cottonwood twigs. These later experiments, however, did not confine the twigs in a tight bottle. They were set up in bell jars, submerged in water as were many of the shoots previously described. It was found that twigs set up at once with eosin, without overcoming saturation deficit, continued to absorb the dye for considerable periods of time. The longer they were submerged in water

and supplied with water at the cut ends before the eosin was applied, the less the dye was absorbed. When left in water sufficiently long, the rise of eosin was only 2–5 mm. in a 24-hour period. It was necessary to take precautions in regard to temperature changes, as a lowering of the temperature during the experiment led to contraction of internal gases and absorption of the dye. With controlled conditions, however, it was demonstrated that the rise of sap in winter twigs is a function of the saturation deficit, and does not depend on a secretory or excretory process.

Discussion

The results obtained in the simple experiments recorded here indicate that sap ascent is controlled, not by the secretion of water by the living cells of the leaf, but by the evaporation of water from the leaf. When evaporation is stopped, and the previously developed saturation deficit is relieved or satisfied, sap ascent ceases. There seems to be no good reason for ascribing to the transpiring cells a special secretory function in connection with the rise of water through the plant. It is true that living cells are necessary to the process of sap rise through prolonged periods of time, although there must be considerable water ascent in dead trees, due to purely physical causes. The main function of living cells is presumably in connection with the conversion of imbibitional forces into osmotic forces, development of osmotic action being necessary to the normal development of a pull upon the water in the tracheal tubes. If the living cells of the leaf are killed, they are then unable to transfer water from cell to cell, and the leaf dries out. The dead cell-wall colloids may for a long time continue a slow water loss, with some water rise, mainly through the stem; but the rapid transpiration stream as it exists in living plants is no longer present.

It does not seem probable that respiratory energy is needed for the accomplishment of water movement up the stems of plants. DIXON proposed this theory to account for the apparent rise of water in the absence of evaporation. It is our opinion that the influence of saturation deficits was not properly obviated in this connection.

BOSE's theory of a hydraulic pumping action of cells in the inner cortical regions of the stems finds no support whatever in this

work. There is no secretion of water from living cells in any case studied. The ascent of sap is due entirely to physical and chemical forces, if we can call osmotic action purely physical and chemical in nature. We prefer so to consider it. If evaporation is the only factor in the development of the forces of sap rise, how are these forces developed and applied to the columns of water in the plant? Some of the essential features of the development of water-lifting forces in the leaf colloids were discussed by one of the writers (**8**) several years ago. It may be profitable to review one or two features of the views previously set forth.

The loss of water from the plant is brought about solely by the kinetic activity of the water molecules themselves. The presence of gases around the plant is a hindrance to water loss, as evaporation occurs much more rapidly in a vacuum than at atmospheric pressure. More than any other gas, water vapor in the atmosphere tends to check water loss from the plant; and the rate of water evaporation is determined mainly by the relative humidity of the air. As this humidity decreases, the atmosphere becomes more and more nearly a water vacuum. Other factors, such as air temperature and barometric pressure, movements of the atmosphere, insolation, etc., influence the degree of water vacuum about the plant, and so modify the rate of water loss. Changes are also wrought in the internal mechanism which may modify the rate of water loss, such as the degree of opening and closing of stomata, changes in the permeability of cell membranes, incipient drying of internal walls, etc. In connection with sap ascent, the environmental situation is exceedingly important, and the degree of water vacuum is very important in determining the amount of pull which can be developed in the leaves.

Fig. 1 indicates diagrammatically the various forces involved in water loss, water retention, and water movement by arrows of different lengths, the magnitude of the force being relative to the length of the arrow. In section *A* the magnitude of the forces of evaporation is represented by an outwardly directed arrow, and the magnitude of the forces exerted by water molecules returning from the air to the colloidal tissues is represented by inwardly directed arrows. It must be evident that the rate of loss at any given moment is related to the difference between these two forces. When there is abundance

of free water in the leaf tissue, as at saturation, the outwardly directed arrow would have its maximum length. The difference between the two forces for loss and gain of water would then fluctuate with the length of the inwardly directed arrows (the magnitude of the forces of hygroscopic absorption of water). If the air is nearly saturated, the inwardly directed forces are nearly as great as the outwardly directed ones, and the difference effective for evaporation is small. But when the air is dry the inwardly directed forces are small, and the difference then effective for evaporation is large.

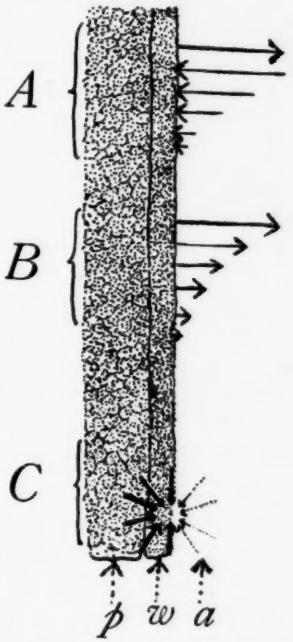

FIG. 1.—Diagrammatic representation of forces of sap rise (outwardly directed arrow, force of evaporation; inwardly directed arrows, forces of atmospheric humidity): *A*, forces of evaporation and humidity; *B*, forces of evaporation, decreasing with development of saturation deficit; *C*, imbibitional force developed on individual particle, which serves as fulcrum for application of force to water movement; *p*, protoplasm; *w*, wall; *a*, internal atmosphere of leaf.

The main concern with reference to the forces of sap rise is what happens in the colloidal material when the tissue begins to lose water faster than it receives it. Then a saturation deficit develops, and the relation of the forces will be as represented in section *B* (fig. 1). With deficit, the outwardly directed forces become smaller and smaller, as indicated by the shorter arrows. Whenever the drying of the tissue reduces the outwardly directed forces to the same size as the inwardly directed ones (*A*), the system is in dynamic equilibrium, and no more water loss would occur; that is, loss and gain would be equal. This occurs rarely except with seeds or other dry organic matter, for in living plants the saturation deficit required to move water in the plant is small. It seems that a vapor pressure deficit of 2 per cent is probably more than enough to lift the sap to the top of the tallest living trees.

Just how is this lifting force created? The answer to this question is to be obtained from a consideration of the relation of the colloidal particles forming the surfaces where the saturation deficit is developing to the water present in connection with those particles. There is a mutual attraction between the molecules of water and the surfaces of the colloidal particles. This mutual attraction results largely from the presence of secondary valencies in the water molecule and in certain of the atoms occurring in the surfaces and interiors of the colloidal particles, particularly whenever the water deficit is pronounced. The water can evaporate from these surfaces only by overcoming all of the attractions occurring on and in the drying colloids.

If we consider the forces in connection with the individual colloidal particles of the membranes, the origin of the pull for water becomes clear. With water deficit, the drier the colloidal particle becomes, the larger is the force which it exerts for the retention of the remaining water. It exerts this force in every direction about it, as indicated in section *C* of fig. 1. This portion of the figure indicates that an individual surface particle exerts an attraction toward water molecules in the atmosphere, as well as toward those farther back in the tissues, away from the atmosphere. But as there is no continuity of material on the atmospheric side, the pull is actually being made effective only for the molecules of water that lie deeper in the tissue. As the surface pull enlarges, the deeper molecules of water, from wall, protoplasm, and vacuole, speedily move toward the surface in response to this colloidal pull. It seems clear that the solid substance of the wall acts as a fulcrum by means of which the forces of the saturation deficit can become effective in moving the water.

The forces developed in the colloids involve capillarity and surface tension, particularly in low-growing plants, in which the surface tensional forces of the menisci in the cell walls may be adequate for the entire phenomena of sap rise. But in taller trees, where the deficit is larger, the forces of adsorption are involved, including molecular, atomic, and ionic surface forces of the ultramicroscopic colloidal particles and water molecules. When the surface particles draw water from those next deeper to replace that lost by evaporation, those particles in turn replace their lost water from still deeper ones, and ultimately from the protoplasmic water, and from the vacuole of the cell. The removal of water from the vacuole by the

protoplast to relieve its own saturation deficit now concentrates the solutes in the cell sap, and converts the imbibitional or adsorption forces into osmotic forces. The osmotic forces are propagated from cell to cell, to the tracheal columns of water, from which the imbibitional and osmotic forces of the border parenchyma cells abstract water. In other words, in reality the saturation deficits and osmotic conditions mentioned merely provide conditions under which the water of the tracheae rapidly migrates into the leaf cells.

We believe that this development of attraction for water in the leaf colloids during evaporation is entirely adequate to account for the ascent of sap in all cases not involving root pressures. Whether evaporation is excessively rapid or extremely slow, the cause of sap movement is held to be due in any case to the development of surface tension, imbibitional and osmotic forces which are in no way related to excretory or secretory activity of the living cells.

Summary

1. These studies are concerned with the ultimate cause of the transpiration stream in the xylem tissue of vascular plants.

2. The current theories of sap rise involve active secretion of water by living cells of the leaves, excepting at times when the evaporation rate exceeds the secretion rate of these cells.

3. Repetitions of DIXON's experiments on sap rise with submerged shoots, and shoots transpiring into a saturated atmosphere without first satisfying the saturation deficit of the shoots, gave results in agreement with his findings. But in shoots in which the saturation deficit was first relieved there was no evidence of secretory activity of the leaf cells, and no significant rise of sap. The phenomena observed by DIXON are therefore believed to have been caused by saturation deficits in the twigs and shoots existing at the time the experiments were performed.

4. Although no experimental evidence is presented, it is believed that downward translocation of dye in the xylem, when leaves on wilty plants are truncated and supplied at the cut ends with eosin, is a consequence of root saturation deficit, which will be relieved from any direction by a supply of free water. It has nothing to do with the normal transpiration or translocation currents in plants.

5. Repetitions of BOSE's bubbler experiments with transpiring

leaves also failed to show any evidence of protoplasmic secretion of water from the leaves. When leaves were coated with vaseline, after proper satisfaction of water deficit, transpiration ceased; and no water was found beneath the vaseline, even after 24 hours.

6. The data presented indicate that there is no reason for assuming any vital action in connection with theories of sap rise, except that of the osmotic action of living cells, in which osmosis ceases with death. Osmotic action is not considered vital action in the usual sense of that term.

7. Sap rise is believed to be caused by the surface tensional and imbibitional action of the cell wall substance, translated into osmotic action in the living cells.

8. A detailed discussion of the development of these forces in connection with the kinetic action of water, pressure and humidity of the atmosphere, the reduced vapor pressure of the leaf colloids, and the consequent pull of the surface forces of the colloids upon the deeper water of the cell is presented.

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REGENERATION IN LEAF CUTTINGS OF IPOMOEA BATATAS

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(WITH NINETEEN FIGURES)

Introduction

There is no general agreement regarding the exact meaning of the term regeneration as applied to plants, or how it differs from the usual methods of asexual propagation. KUPFER (5) believed the word ought to be limited to those cases in which an organ is formed at a place or under conditions in which it would not normally be formed. This seems to be as simple a definition as has been given, and explains, at least in a general way, how regeneration differs from asexual propagation.

Many have attempted to discover what there is in a detached part of a plant that gives it the power of regeneration. The early investigators in attempting to explain the phenomenon used parts of plants not normally producing shoots or roots. MCCALLUM (6) stated that the leaves of many plants possess the power of organizing new shoot primordia. He showed a rooted begonia leaf and mentioned other plants that may be regenerated from leaves. VÖCHTING (9), cited by KUPFER (5), expressed the opinion that the leaf in which the power to regenerate is lacking would upon investigation prove to be the exception rather than the rule. KUPFER, however, experimented with leaf cuttings of 82 species and failed to confirm this. Roots were formed by 61 species while only 12 gave rise to shoots. She failed to obtain plants from leaf petiole cuttings of any species. She found that the base of the petiole of Irish potato leaf cuttings enlarged and served as a place of storage. BROUN (2), LOEB (4), and REED (7) worked with regeneration of *Bryophyllum* plants from leaves. LOEB's experiments tended to show that leaves of *Bryophyllum* would not produce roots and plants unless the leaf were detached or the transpiration stream between the leaf and other parts of the plant severed. BROUN showed that leaves of *Bryophyllum* would produce roots and shoots without their being detached or the trans-

piration stream being severed. REED made observations similar to those of BROUN. He also found that the base of the petiole of detached leaves of *Bryophyllum* rooted, and that the leaves with the rooted petioles lived for several months without producing shoots. He did not believe, like LOEB, that one part of a plant necessarily inhibited the growth of other parts. He noted that the meristematic tissue in the notches of the leaves of *Bryophyllum* where shoots arise contained much pigment. CHILD and BELLAMY (3) showed that under certain conditions a leaf of *Bryophyllum* might inhibit development of a bud. The early investigators failed to reach an agreement as to the cause of regeneration of roots and shoots from parts of plants not normally producing these parts. They seemed to agree in a general way, however, that leaf cuttings have more power to regenerate roots than shoots.

More recently investigators have conducted experiments to determine the root and shoot responses from different kinds of shoot cuttings when placed under similar and under different environments. The general conclusions from such researches are that the variety, chemical composition of the cutting, presence or absence of foliage, heat, light, moisture, or nutrients in the propagation medium or treatment of the cutting with chemicals may influence the amount and nature of the root and shoot response.

REID (8) found that shoot cuttings of the tomato sometimes tended to do what the plant had been doing when the cuttings were taken.[1] ZIMMERMAN and HITCHCOCK (10) observed that the leaf petioles remaining on shoot cuttings of dahlias sometimes became enlarged and served as a place of storage; moreover, the petioles of leaf cuttings often rooted when placed in a propagation medium. They found that shoot cuttings of dahlias taken at various seasons showed peculiar varietal responses to changes in day length. They also found that the length of day influenced the character of the root system; short days encouraged storage roots and long days, fibrous roots.

[1] After this manuscript was prepared, a bulletin appeared (PRIESTLEY, J. H., and SWINGLE, C. F., Vegetative propagation from the standpoint of plant anatomy. U.S. Dept. Agric. Tech. Bull. 151), citing and discussing other literature treating of the effect of the plant upon its regenerations.

Sweet potato plants are usually obtained by bedding the developed roots or by rooting vine cuttings. Plants can be produced by cutting sweet potatoes into pieces and planting somewhat as Irish potatoes. Plants are sometimes obtained from seed in hot climates, where there is a long growing season. KUPFER found that thin cross-sections of peeled sweet potato roots, when planted in sand, callused on the cut surfaces and regenerated roots from near the center on both surfaces. She also planted sweet potato peelings from which buds had been removed and obtained roots; during the two months of the experiment, however, none of the parts in which shoot buds were lacking regenerated buds. BEALS (1) removed one end of an enlarged sweet potato root by a transverse cut and found that a shoot regenerated out of the cambium at the cut surface.

The writer has not found any literature which refers to the regeneration of roots or shoots by sweet potato leaves. In June 1929 a few sweet potato leaves were placed in a propagation bed and roots began to develop within 3 days. This immediately raised the question as to what part or parts of the leaf would regenerate roots. It also raised the question whether a new plant would arise from the leaf cutting, and, if so, what would be the location of the adventitious bud which produced the new shoot. Different types of sweet potato leaf cuttings were taken, rooted, and observed until plants or potatoes or both were produced. Observations made on these cuttings are reported in this paper.

Procedure

Cuttings were taken on bright sunny days not earlier than noon, usually not until late afternoon. Cuttings were taken at this time because of the general belief that the leaf is more likely to contain a large amount of carbohydrate material after it has been exposed to light for some time, and that soft wood cuttings root better when sufficient carbohydrate material is present.

Cuttings from four different varieties were observed. The pigmentation other than that due to chlorophyll (pigment in this paper does not refer to chlorophyll) varied in different varieties. At least three varieties were included in some of the types of cuttings studied. There were seven general types of cuttings observed; some of them

of one type were further modified when transferred from the propagation bed. The types of cuttings are described as follows:

1. An entire leaf carefully removed from the vine so that no part of the stem was left on the petiole. Some of these cuttings were further modified by the removal of the leaf blade at the time the cuttings were potted.

2. A leaf blade and half of the leaf petiole.

3. A leaf with the blade and petiole reduced one-half.

4. A leaf blade and a portion of the petiole, prepared by removing all of the petiole except the enlarged portion where it united with the leaf blade.

5. Like type 4, except that the entire petiole and the thickened part of the leaf blade where the leaf and petiole unite were removed, the remainder of the leaf blade being used as the cutting.

6. Terminal one-half of the midrib and one-fourth of the leaf blade, prepared by cutting a leaf blade transversely into two parts of about equal leaf surface. The terminal half of the leaf blade was used as the cutting, after it was further modified by removing enough of the leaf surface to allow the basal part of the midrib to extend into the sand.

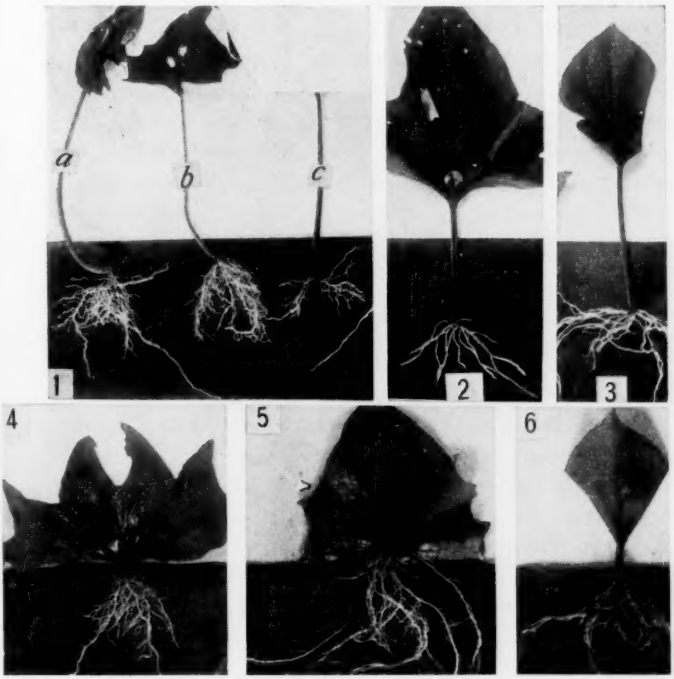
7. Leaf petiole.

Figs. 1–6 illustrate types of cuttings 1–6 respectively, as they appeared when taken from the propagation bed and prepared for potting.

The cuttings were moistened with water as soon as taken, carried to the greenhouse, and placed in sand to root. The propagation bed was equipped with a frame and glass cover which were used to control the humidity. For the first 2 or 3 days after taking, the cuttings were sprinkled with water three or four times during the day to maintain a rather high humidity. Very frequent sprinkling was not practiced after that time. The glass cover was removed at night to allow ventilation.

Some of the cuttings were ready to be taken out of the sand in 5 or 6 days, but in general cuttings were left in the bed until practically all that were made at any given date were well rooted, which required about 10 days. The rooted cuttings were transferred to small clay pots containing potting soil. (At this time it was not expected

that the experiment would be continued beyond the time when a shoot appeared, therefore the pots containing the rooted cuttings were set in available greenhouse space without special regard to light,



FIGS. 1–6.—Fig. 1, representative cuttings of entire leaves: *a*, non-pigmented; *b*, pigmented; *c*, leaf blade and petiole with leaf blade removed at time cutting was potted. Fig. 2, leaf blade and half of petiole. Fig. 3, reduced leaf blade and half of petiole. Fig. 4, reduced leaf blade and enlarged part of petiole where it unites with leaf blade. Fig. 5, leaf blade with part of base removed. Fig. 6, portion of leaf blade and part of leaf midrib.

heat, moisture, or ventilation. Actually, the plants were in shade most of the time.) Some were transplanted from the pots to the field. At the end of the experiment the older cuttings and their regenerations were growing in 8-inch pots, and the younger ones, or those taken last, were in 4-inch pots. About a month before the experiment was dis-

continued all pots were transferred to the open, and the cuttings left in them. Cuttings which were potted last were not repotted, unless taken up to photograph.

Results

Observations were made at frequent intervals during the progress of the experiment, and all plants were examined when the experiment was being discontinued October 3 and 4. Results obtained from pot-grown plants are shown in table I. It will be seen that all of the cuttings of type 1 (which included varieties with a wide range in pigmentation) had not produced shoots when the experiment was discontinued. Most of the cuttings in this group failed to regenerate shoots from fibrous roots. Fig. 1 illustrates some of the cuttings of this type, photographed after they had been in the propagation bed 12 days. It will be seen that all cuttings did not behave alike. One kind of response, characteristic of the variety that contained no pigment, was for the leaf blade to die before a shoot was regenerated (fig. 1*a*) or soon after (fig. 9*c*), or to remain alive while the lower part of the petiole enlarged (fig. 11). One cutting with enlarged petiole was kept under observation after the others had been discontinued; it finally produced a shoot from the callus of the petiole. Another kind of response, typical of a variety with some pigment, was for the cutting to produce a potato and to form shoots from the callus on the enlarged base of the petiole and from the potato at about the same time. A cutting of this type is shown in fig. 7, with a shoot from the callus and from the potato at *b* and *c* respectively. The variety with much pigment in the leaves tended to form shoots rather quickly from the callus, and in some instances the parent leaf remained alive until its shoots had made considerable growth. There was a general tendency for cuttings of this variety to produce more than one shoot from the callus on the petiole. Fig. 8*a* illustrates cuttings of this variety with a shoot (*b*) just as it was developing from the callus 18 days after the cutting was taken. The same parent leaf is shown in fig. 9*a* as it appeared 18 days later, after two other shoots had appeared. This leaf was alive when its shoots had reached 48, 14, and 6 inches in length respectively.

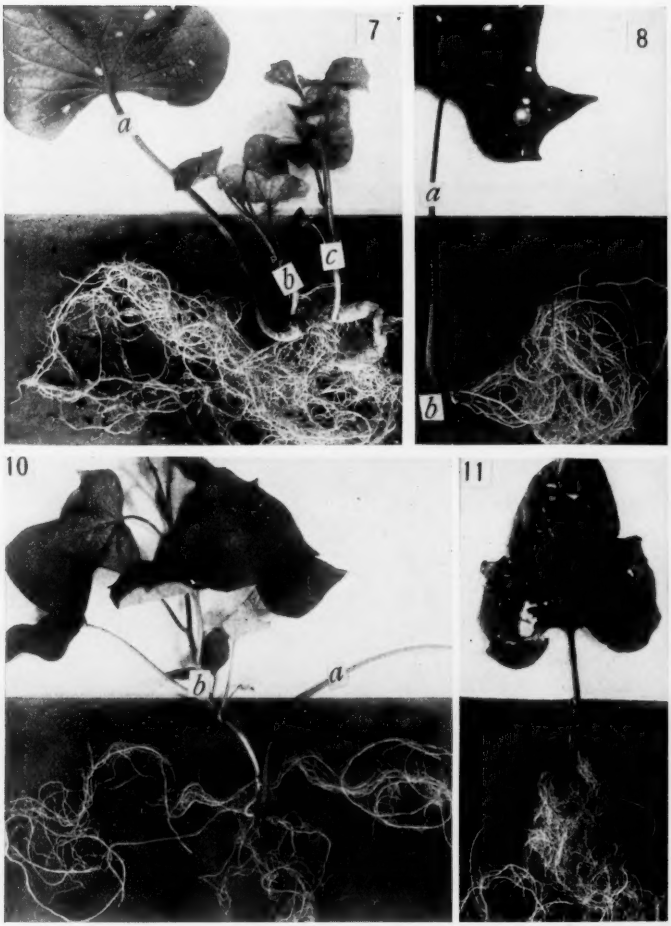
In the cutting of type 1 (fig. 1*c*) the leaf blade was removed at the time of potting, to determine whether the rooted petiole could re-

TABLE I

GROWTH OF DIFFERENT TYPES OF SWEET POTATO LEAF CUTTINGS

TYPE OF CUTTING	NUMBER	DATE CUT (1929)	DATE OF FIRST SHOOT (1929)	NO. PRODUCING SHOOTS	AVERAGE NO SHOOTS PER CUTTING	ORIGIN OF SHOOT*				CHARACTER OF ROOT SYSTEM			CONDITION OF PARENT LEAF	
						No. from callus	No. from between callus and potatoes	No. from potatoes	No. from small or fibrous roots	No. producing small or fibrous roots	No. producing enlarged or stringy roots	No. producing potatoes	No. dead	No. fair to good condition
1 (Entire leaves):														
Pigment ranging from much to none	21	6/21	7/21	17	1.20	16	4	5	0	2	8	11	14	7
Pigment in few leaves	11	7/6	9/18	2	0.18	0	0	0	2	5	6	0	0	11
Leaf blade removed when potted; some pigment	2	6/21	7/3	1	0.50	1	0	0	0	1	1	0	0	2
2 (Leaves with 1/2 petiole removed when cuttings taken):														
No pigment	11	6/30		0	0.00	0	0	0	0	9	0	2	0	11
No pigment	2	7/6		0	0.00	0	0	0	2	2	0	0	0	2
Some pigment	10	7/6	9/8	6	1.00	2	0	8	0	2	8	0	0	10
3 (Leaves with blades and petioles reduced at cutting):														
Some pigment in all cuttings	13	6/30	8/23	5	0.62	2	0	0	7	10	3	0	1	12
4 (Leaf blades with petiole removed except enlarged part where petiole unites):														
All leaves had much pigment	12	6/30	8/3	12	1.33	13	0	2	1	5	5	2	3	9
All leaves had much pigment	12	7/6	8/30	9	0.83	0	0	2	1	2	8	2	2	10
5 (Leaf blades with entire petiole removed including enlargement where petiole unites):														
All leaves including pigment	6	8/3	9/30	2	0.33	1	0	1	0	0	5	1	0	6
6 (Small part of leaf blades from terminal part of leaves with part of midrib):														
Leaves containing some pigment	10	8/3	10/4	1	0.10	0	0	0	1	9	0	1	0	10
7 Leaf petioles	10	6/21	Cuttings of this type observed only until roots formed.											

* In several instances it was almost impossible to ascertain whether shoot originated from callus or potato or some point between.



FIGS. 7, 8, 10, 11.—Fig. 7, *a*, parent leaf of variety containing some pigment; *b*, shoot arising from enlarged callus; *c*, shoot arising from potato. Fig. 8, *a*, parent leaf of variety containing much pigment; *b*, young shoot appearing from callus. Fig. 10, *a*, parent petiole from which entire leaf blade was removed on potting; *b*, shoot regenerated from callus on base of petiole. Fig. 11, leaf blade and half of petiole, showing base of reduced petiole enlarged and serving as place of storage.

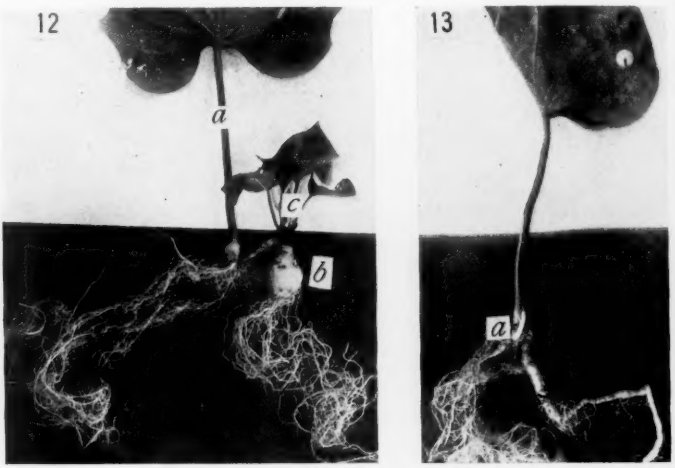
generate a shoot with this removal. From table I it will be seen that one of the two cuttings of this type produced a shoot and the other did not. Fig. 10 illustrates the cutting with its shoot as it appeared 68 days after the cutting was made. When examined October 3, the cutting which did not produce a shoot apparently had changed very little from the time it was potted, except that the older roots appeared to be breaking down and new ones forming. The parent leaf petiole was still alive and appeared healthy. The cuttings of type 1 grown in the open behaved much as did those in pots, except that the shoots which they regenerated tended to make more vine growth. The length of shoots of one of these cuttings totaled 50 feet of vine when the experiment was discontinued.

FIG. 9.—*a*, parent leaf (same as fig. 8) after three shoots had appeared; *b*, parent leaf of variety producing relatively few shoots; *c*, parent leaf that died soon after shoot appeared.

It will be noted from table I that cuttings of type 2, from the variety without pigment, had not produced shoots when the experiment was discontinued. All of the non-pigmented leaves were alive at this time. The petioles of some of the cuttings of this type split in the propagation bed; such petioles healed and served as a place of storage. The petioles that did not split also enlarged and served for storage (fig. 11). Cuttings of type 2 from a variety with some pigment tended either to produce potatoes from which shoots developed rather quickly (fig. 12), or to produce a potato directly from the petiole and then to form a shoot (fig. 13). Cuttings of the variety with heavy pigment produced plants rather quickly and freely from the callus of the petiole (fig. 14). The basal portion of the petiole of this variety did not tend to enlarge and serve as a place of storage.

Seven out of nine of the shoots produced by cuttings of type 3 originated from fibrous roots, and the other two from the callus. There was little or no tendency for the reduced petiole to enlarge and serve as a place of storage, or for potatoes to be formed before shoots.

The variety represented in this type was not represented in type 2, which may account for the difference in behavior of the cuttings of types 2 and 3. This type of cutting is illustrated in fig. 15, showing at *b* a shoot arising from a fibrous root which came to the surface. Fig. 15 also shows a young shoot developing at *c* on the same root nearer the parent leaf. A younger shoot is developing at *d* from another fibrous root of the same plant.

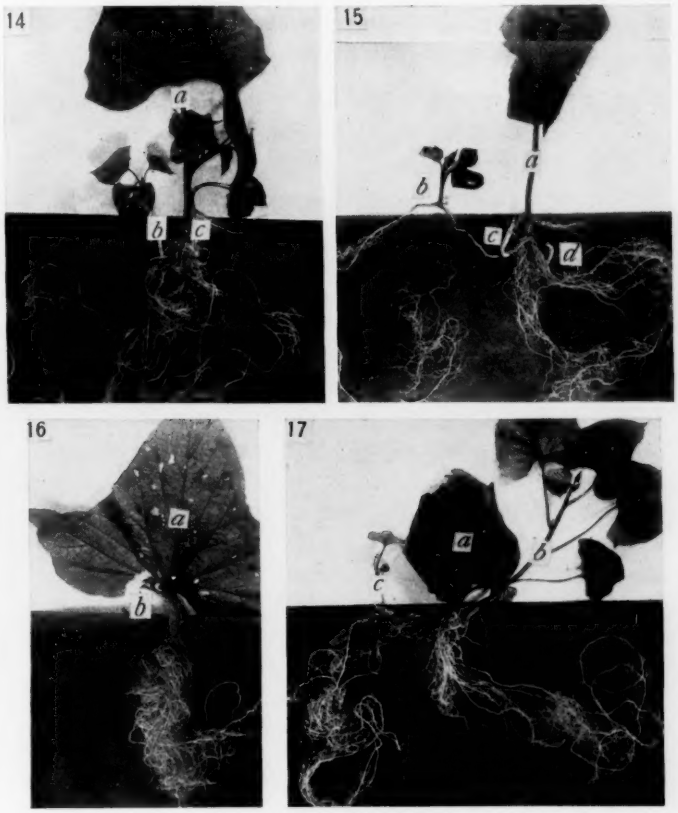


FIGS. 12, 13.—Fig. 12, *a*, leaf blade and half of leaf petiole; *b*, potato; *c*, shoot produced from it (photograph taken 2 months, 8 days after cutting; potato weighed 17 gm., green weight). Fig. 13, same behavior as fig. 12, except that potato was produced directly from end of reduced petiole; *a* arose from point near where potato grew out.

Type 4 gave the highest number of shoots per cutting. Nineteen of the 25 shoots produced by cuttings of type 4 arose from the callus (figs. 16, 17), two from fibrous roots (fig. 17*c*), and four from potatoes. Table I shows that relatively few of the cuttings of type 5 had produced shoots when the experiment was discontinued. This can probably be explained by the cuttings being taken later than the others. A cutting of this type is shown in fig. 18 with a shoot (*b*) arising from the callus. A cutting of the same type is shown in fig. 19, with the leaf blade partially torn apart to show a greater root

development from the midrib than from smaller veins. A potato was developed on the midrib. The potato produced a shoot.

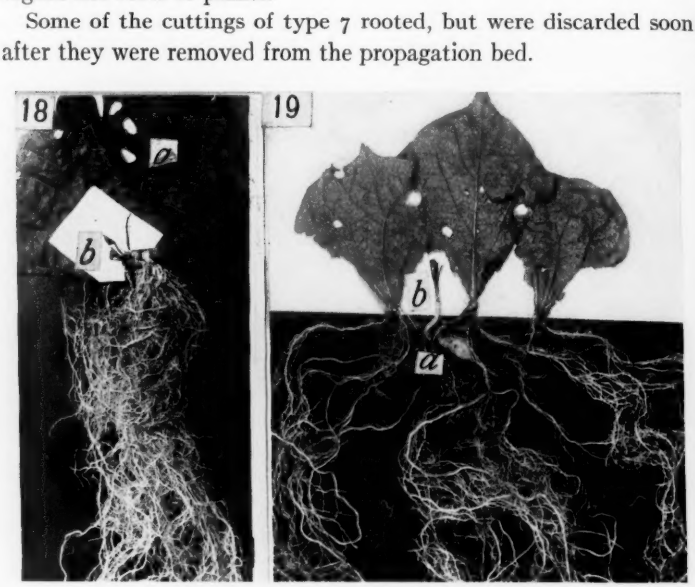
One cutting of type 6 produced a potato directly on or out of



FIGS. 14–17.—Fig. 14, *a*, parent leaf of variety containing much pigment; *b*, *c*, shoots arising from base of reduced petiole. Fig. 15, *a*, parent leaf with reduced leaf blade area and reduced leaf petiole; *b*, shoot regenerated from fibrous root that came to surface; *c*, shoot regenerated from same fibrous root as *b*; *d*, young shoot developing on another fibrous root. Fig. 16, *a*, leaf blade and terminal or enlarged part of petiole; *b*, shoot regenerated from callus. Fig. 17, *a*, same type as *a* of fig. 16; *b*, shoot arising from callus; *c*, shoot arising from small root.

the base of the reduced midrib. A cutting of this type regenerated a shoot out of the base of the reduced midrib. Cuttings of type 6 were used to determine how small a leaf area might be used to regenerate roots or plants.

Some of the cuttings of type 7 rooted, but were discarded soon after they were removed from the propagation bed.



FIGS. 18, 19.—Fig. 18, *a*, leaf from which petiole and part of leaf blade were removed on cutting; *b*, origin of shoot. Fig. 19, same type as fig. 18; parent leaves partially torn apart to show development of roots from different regions: *a*, potato; *b*, shoot.

Discussion

Sweet potato leaf cuttings from different varieties behaved differently. It cannot be stated from the data whether the behavior of different cuttings was due to variety, to amount of pigment in the cutting, or to some other cause. The effect of the stage of growth of the plant from which cuttings were taken on the behavior of the cuttings, as reported by REID (8) for tomatoes, was not ascertained. REED (7) kept leaves of *Bryophyllum* with rooted petioles in sand for 6 months without the production of shoots: if the rooted petioles enlarged and served as a place of storage as did some of the sweet

potato petioles he failed to mention it. The failure of rooted leaves of *Bryophyllum* to regenerate shoots was used by REED (7) to question certain of LOEB'S (4) theories of the inhibitory effects of one part on another. The behavior of cuttings of different varieties of sweet potato leaves suggests that this plant might be of value for work such as LOEB carried on with *Bryophyllum*. A comparative study of varieties using the petiole as a place of storage and those that early differentiate a bud from the petiole might have more nearly harmonized the ideas of LOEB and REED concerning the inhibition and regeneration of shoots from leaf cuttings.

The petiole which regenerated a shoot after the entire leaf blade was removed (fig. 10) shows that rooted petioles of that variety are not necessarily dependent on leaf blades for power to regenerate a shoot. KUPFER (5) failed to find any species in which an isolated petiole formed a shoot. In the work reported in this paper petioles without an attached leaf blade formed a rather limited root system, but were not observed long enough to determine whether they would develop shoots.

In type 3 the appearance of shoots on small roots at different distances from the parent leaf, as well as the appearance of more than one shoot on a small root, is interesting in connection with the statement by CHILD and BELLAMY (3) that a leaf may inhibit a bud and that one part may inhibit another within certain distances. It is also of interest to note that enlargement of the petiole was not found with this group of shoots. The appearance of the first shoot from a root that came to the surface is of interest in connection with the fact that REED (7) found that the *Bryophyllum* leaf produced plants much sooner in the dark.

Under the conditions of this experiment, there appeared to be a quicker shoot response from pigmented cuttings. There also appeared to be a quicker shoot response from leaf cuttings with reduced or no petioles than from those with entire petioles. It is suggested that the cuttings with more petiole had the power to use the petiole for storage, and in this way tended to inhibit shoot formation.

Since there appears to be a rather definite relationship between root development from the midrib and that from other veins (fig. 19), it is suggested that this type of cutting might be used to measure

the amount of material manufactured by the different parts of the leaf blade.

Summary

1. Six different types of sweet potato leaf cuttings were rooted and observed until plants were regenerated. A seventh type was discarded after roots regenerated but before shoots were formed.

2. All types of cuttings regenerated roots quickly.

3. When the leaf blade was removed from a rooted leaf cutting the remaining petiole had the power to regenerate a shoot.

4. Leaf blades or parts of leaf blades with or without the petiole regenerated roots and shoots.

5. Cuttings regenerated shoots from the callus on the petiole, from fibrous roots, from fleshy roots, or from more than one of these points.

6. Leaf blade cuttings produced most of the roots out of the midrib and veins, the former showing the greater development.

7. In some cuttings potatoes were produced directly on or out of the petiole or reduced midrib.

8. Entire petioles or reduced petioles of cuttings from a non-pigmented variety enlarged and served as a place of storage. These failed to differentiate shoots or did so very slowly.

9. Leaf cuttings of a variety with a small amount of pigment tended either to produce a potato before a shoot differentiated or to differentiate shoots from fibrous roots.

10. Leaf cuttings of a heavily pigmented variety produced shoots rather freely either from the callus on the petiole or from small roots.

ALABAMA EXPERIMENT STATION
AUBURN, ALA.

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FLORAL MORPHOLOGY OF LYONOTHAMNUS FLORIBUNDUS

JOSÉ B. JULIANO

(WITH TWENTY-NINE FIGURES)

Introduction

There is a conflict of opinion as to the systematic position of *Lyonothamnus floribundus* Gray, a flowering tree endemic to the Channel Islands off the coast of California. GRAY (**8**), as well as GREENE (**9**) and JEPSON (**14**), believed that despite its saxifragaceous fruit it should be placed in the Rosaceae. SARGENT (**22**) at first considered it one of the Saxifragaceae, but later (**23**) accepted GRAY's opinion. BRITTON (**2**) removed it from the Saxifragaceae and put it under the closely allied family of Cunoniaceae. This investigation was undertaken at the suggestion of Professor LEROY ABRAMS, in the hope that the internal morphology of the flower might shed light on these conflicting views.

The material used was gathered at frequent intervals, from May 16 to August 16, 1929, from two healthy trees, one of which grows in the garden of Professor DURAND on the Stanford University campus, the other on the campus of the University of California.[1] A chromo-acetic solution, prepared according to the formula given by CHAMBERLAIN (**3**), Bouin's fluid (aqueous and alcoholic), and formalin-acetic-alcohol were employed as fixatives. The material was imbedded in the usual way, and sections were cut 10–15 μ in thickness. Most of the preparations were stained with Haidenhain's iron-haematoxylin and counterstained with aniline orange gold dissolved in clove oil. Flemming's triple stain and safranin-light green combination were utilized to advantage.

Investigation

FLOWER

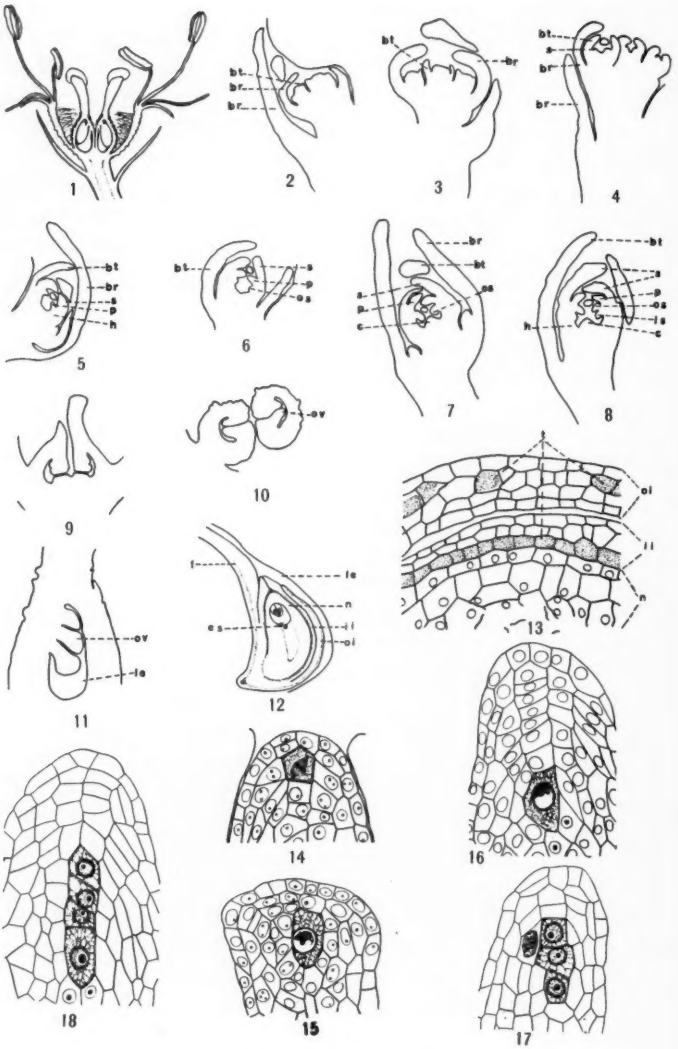
The perfect small white flower, which is borne in a much branched and rather broad terminal panicle, arises from the ultimate branches

[1] I wish to express thanks to Mr. JOHN W. GILLESPIE, who collected some of the material from the tree at Stanford, and to Mr. ARTEMIO V. MANZA, who made periodic collections for me at Berkeley.

of the inflorescence. Each flower is subtended by two or three, sometimes four, small and acute persistent bractlets (fig. 1). The five sepals, which are inserted on a circular 5-lobed hypanthium, are nearly triangular, apiculate, and also persistent. The hypanthium is tomentose on its outer surface, and woolly hairs which persist to maturity of the fruit arise from the inner surface. JEPSON (**14**) mentions the deciduous character of the calyx lobes of *Lyonothamnus*, but my study indicates that they remain on the mature fruit, where they become rather brittle. On this hypanthium are five petals which alternate with the calyx lobes. The petals are nearly always orbicular, sessile, and white. Within the corolla is an outer whorl of ten stamens, one opposite each petal and sepal, and an inner whorl of five stamens, one opposite each sepal. The number of stamens is irregular, however, and varies from thirteen to sixteen. Inclosed by the hemispherical hypanthium are two pistils, each bearing a rather club-shaped style and capitate stigma, which arise from the abbreviated floral axis (receptacle). Three pistils are sometimes present. The ovaries are mono-carpellary and usually contain four ovules, although six are not uncommon. The anatropous oblong ovules, which are suspended on long funiculi, possess superior micropyles at their maturity, and are borne one above the other on the ventral parietal placenta in two distinct series.

The individual flower starts as an emergence from the axil of a bract. Usually three floral primordia arise simultaneously (fig. 3), the middle one always developing much more rapidly than the lateral ones. The first members to differentiate are the bractlets, which arise as lateral mammillate protrusions (fig. 2). These soon elongate and cover the floral axis (fig. 4). As soon as the bractlets are well differentiated the five sepals develop simultaneously from the periphery of the receptacle. Within the calyx whorl and alternating with the young sepals, the five petals next appear simultaneously as mammillate humps (fig. 5). There follows an elongation of the tissue on which the sepals and petals are borne. Because of the elongation of the hypanthium, the apex of the floral axis becomes a depression within it.

After the hypanthium has elongated somewhat, the primordia of the outer whorl of stamens appear, one opposite each petal and sepal,



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just within the petals (fig. 6). As the outer whorl of stamens starts to lengthen, there begins the development of two, sometimes three, hemispherical carpellary humps (fig. 7) from the apex of the receptacle. The inner whorl of five stamens, one opposite each sepal (fig. 8), develops last. In other words, development of the floral organs in *Lyonothamnus* is not acropetal, and they arise as follows: bractlets, sepals, petals, outer stamens, carpels, and inner stamens. COULTER and CHAMBERLAIN (4) cite that HOFMEISTER records the development of the carpels in *Rosa*, *Potentilla*, and *Rubus* before those of the stamens have reached their full number, a feature similar to that existing in *Lyonothamnus*. On the other hand, in *Astilbe japonica* WEBB (30) finds a similar development of the carpels before the last whorl of stamens is formed, but in this plant the outer whorl of stamens is the last to be developed. The sequence of appearance of the floral parts, therefore, is not a good criterion for determining whether *Lyonothamnus* should belong to the Rosaceae or to the Saxifragaceae.

FIGS. 1–18.*—Fig. 1, longitudinal section of mature flower. Fig. 2, portion of median section of branch of inflorescence showing floral primordia at axil of bract; bractlets beginning to differentiate. Fig. 3, older branch showing floral primordia with bractlets already differentiated. Fig. 4, much older branch showing differentiation of sepals in floral primordia. Fig. 5, longitudinal section of single flower with bract, bractlets, sepals and petals (note growth of hypanthium). Fig. 6, longitudinal section of older flower showing beginnings of primordia of outer whorl of stamens. Fig. 7, longitudinal section of much older flower showing beginnings of carpel. Fig. 8, longitudinal section of flower after differentiation of parts. Fig. 9, longitudinal section of pistils showing origin of ovules. Fig. 10, transverse section of ovaries showing two series of ovules. Fig. 11, longitudinal section of older ovary showing three ovules in single series. Fig. 12, diagram of longitudinal section of mature ovule showing relative lengths of megagametophyte before and after absorption of chalazal nucellar tissue; two antipodal cells (blackened) still persisting. Fig. 13, portion of longitudinal section of integuments and nucellus of mature ovule. Fig. 14, nucellus with archesporial cell dividing. Fig. 15, nucellus after formation of primary parietal cell and megaspore mother cell. Fig. 16, deep-seated megaspore mother cell in synapsis (note two conspicuous nucellar cells below spore mother cell). Fig. 17, megaspore mother cell and two lower nucellar cells exhibiting same staining capacity, size of nuclei, and amount of cytoplasm. Fig. 18, linear tetrad of four megaspores; chalazal megaspore enlarging; two basal nucellar cells well differentiated.

* Designations are as follows: antipodals *an*, basal apparatus *ap*, bract *br*, bractlets *bt*, carpels *c*, cotyledon *cl*, embryo *em*, endosperm *en*, hypanthium *h*, hypostase *hy*, inner integument *ii*, inner stamens *is*, locule *le*, megagamete *e*, megagametophyte *es*, nucellus *n*, outer integument *oi*, outer stamens *os*, ovule *ov*, petals *p*, plumule *pl*, polar nuclei *pn*, pollen tube *pt*, radicle *r*, raphe *re*, tanniferous cells *t*, seed coat *te*, vascular bundle *vb*, and zygote *ze*.

MEGASPORANGIUM

The development of the megasporangium has been traced and shows nothing novel. Four (figs. 9, 10), sometimes six (fig. 11), ovules emerge as mammillate protuberances on the parietal placentae, and these soon elongate laterally and sometimes obliquely downward into the loculus of the ovary, forming the nucellus of the nascent ovules. Integuments are developed in basipetal succession. At maturity of the ovule the outer integument reaches only a little beyond the level of the apex of the nucellus (fig. 12), and consists of four layers of cells (fig. 13). Underneath its outer epidermis is a layer of tanniferous cells. The inner integument completely covers the nucellus and consists of three layers of cells. Toward the micropyle the hypodermal cells of the inner integument divide longitudinally and an increase in breadth results. The innermost layer of cells of the inner integument possesses tanniferous substance, and this layer delimits the nucellus to the chalaza.

An obturator is formed by a great number of the rosaceous genera, especially those belonging to the Spiraeoideae, Pomoideae, and Prunoideae (**13**). This obturator, which develops simultaneously with the ovules, is morphologically considered by PÉCHOUTRE (**20**) to be an abortive ovule, and on this assumption he explains its absence in most if not all of the "Spirées." This structure is unknown in the Saxifragaceae, except for *Francoa appendiculata* in which GÄUMANN (**7**) reports the development of a "Funiculuswarze" which functions as an obturator. No obturator is formed in *Lyonothamnus*, which produces a variable number of ovules. It is interesting to note, however, that at the time the megaspore mother cell is formed, there is almost always a faint differentiation of a few cells of the funiculus of the uppermost ovule just close to the ovarial wall. A few of the epidermal cells become palisade-like and stand out conspicuously from the rest of the cells. This differentiation is so slight that one is apt to overlook it. Aside from this feeble growth of a few funicular cells of the uppermost ovule in each series, there is nothing suggesting an obturator in *Lyonothamnus*. In this respect the ovule is saxifragaceous rather than rosaceous in nature.

As soon as the inner and outer integuments have been fully differentiated, and long before the micropyle becomes superior owing to

the growth of the funiculus, there is the differentiation of a one-celled archesporium (fig. 14). This archesporial cell divides periclinally, giving rise to a primary parietal cell and a megaspore mother cell (fig. 15). The development of a many-celled archesporium, a character true to a majority of the Rosaceae (13), has not been observed in *Lyonothamnus*.[2] Among the Saxifragaceae there is generally found a development of a one-celled archesporium: *Saxifraga* (15, 19, 24), *Heuchera* (19), *Chrysosplenium* (15, 28), and *Ribes* (6, 11). The archesporium may function directly as a megaspore mother cell in *Parnassia palustris* (19), and no parietal tissue is consequently developed. Exceptions to this are to be seen in the many-celled archesporium of *Astilbe japonica* (30), *Philadelphus* (7), and *Ribes nigrum* (29). On the whole one may say that a several-celled archesporium is generally found in the Rosaceae and a single-celled archesporium in the Saxifragaceae. That of *Lyonothamnus*, therefore, is saxifragaceous rather than rosaceous in nature.

Considerable parietal tissue is developed while the megaspore mother cell is in synapsis (fig. 16), so that the megaspore mother cell comes to lie deeply sunken in the nucellus. Among the Rosaceae, PÉCHOUTRE (20) finds the formation of two to several layers of parietal tissue, and this is accompanied by periclinal divisions of the nucellar epidermis which forms a covering (the "coiffe épidermique") over the latter. In the Saxifragaceae some genera have the development of a rather thick parietal tissue, while others produce none at all. The Saxifragaceae never form a "coiffe épidermique." *Lyono-*

[2] A survey of the literature on this question shows that multicellular and unicellular archesporia are formed as follows in the two families:

ROSACEAE
Multicellular archesporium:
Pomoideae: *Eriobotrya, Pirus, Malus, Amelanchier* (13).
Rosoideae: *Kerria, Rhodotypus, Rubus, Fragaria, Dryas, Geum, Alchemilla, Sanguisorba, Agrimonia, Rosa* (13), and *Potentilla* (24).
Prunoideae: *Prunus* (13).
Unicellular archesporium:
Neurada (18), *Amygdalus campestris* (15), *Prunus* (21).

SAXIFRAGACEAE
Multicellular archesporium:
Astilbe japonica (30), *Philadelphus* (7), *Ribes* (29).
Unicellular archesporium:
Saxifraga (15, 19, 24), *Heuchera* (19), *Chrysosplenium* (15, 28), and *Ribes* (6, 11).

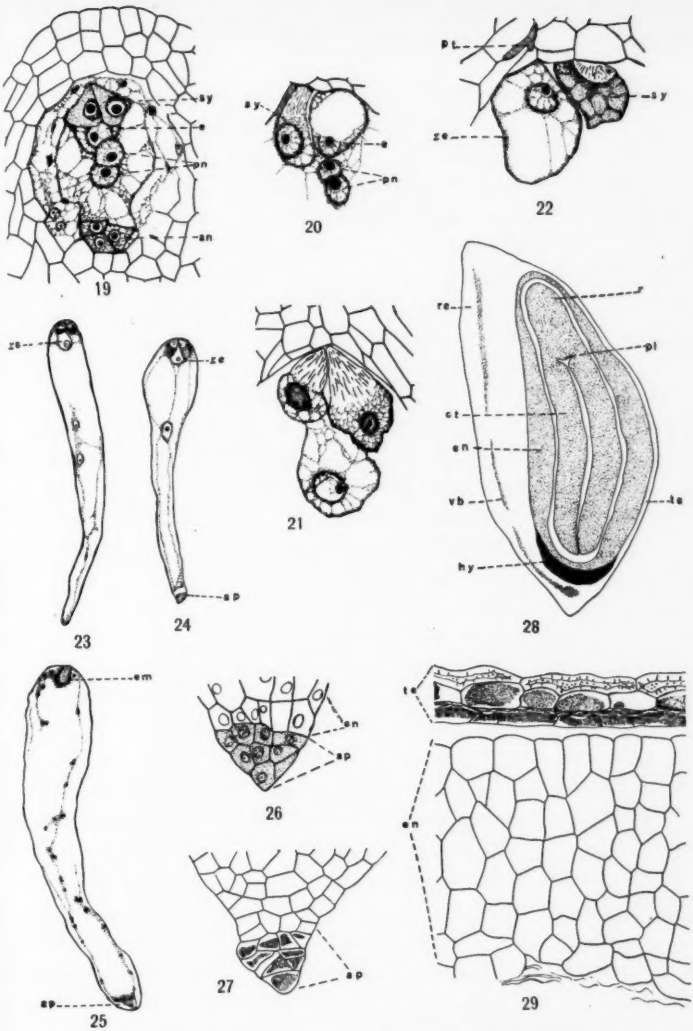
thamnus, which develops a rather thick parietal tissue without an epidermal cap, has therefore a nucellus that is more saxifragaceous than rosaceous in nature.

Although a single megaspore mother cell is developed in *Lyonothamnus*, one usually finds some of the nucellar cells adjacent to the megaspore mother cell exhibiting characters typical of the latter. In fig. 17, for example, just beneath the megaspore mother cell are two nucellar cells which exhibit the same staining capacity, size of nuclei, and amount of cytoplasm. To the left is another nucellar cell undergoing vegetative division. These conspicuous nucellar cells never give any indication of functioning as spore mother cells, although they may remain evident up to the binucleate stage of the megagametophyte. Although *Lyonothamnus* develops a saxifragaceous archesporium, this differentiation of several of its nucellar cells may suggest that it is not far removed from the Rosaceae, and that it may have come from a rosaceous ancestor which develops many megaspore mother cells either from a single or multicellular archesporium.

MEGAGAMETOPHYTE

By two successive divisions the megaspore mother cell gives rise to a linear tetrad of four megaspores (fig. 18), the chalazal, the third megaspore of which rarely becomes functional. A normal 7-celled megagametophyte is formed by three successive divisions of the nucleus of the functional megaspore. In the mature megagametophyte (fig. 19) the egg apparatus (megagamete and two synergids) and two polar nuclei occupy its micropylar end. The megagamete usually lies between the synergids. At the chalaza the three antipodal cells are formed. These persist for some time after their formation and have a dense cytoplasm and distinct nuclei. They disappear during elongation of the megagametophyte and before fertilization.

Soon after the formation of the 7-celled megagametophyte, there is rapid destruction of the nucellar cells at the chalazal end (fig. 12). Accompanying this absorption of the chalazal tissue is an elongation of the gametophyte, which assumes a cylindrical or tapering shape with its swollen end directed toward the micropyle. As a result of this elongation the antipodals come to lie to one side before they



FIGS. 19–29.—Fig. 19, megagametophyte showing migration of chalazal polar nucleus and cells cutting off. Fig. 20, egg apparatus and polar nuclei; one of synergids not shown (note filiform apparatus at base of synergid). Fig. 21, egg apparatus before fertilization. Fig. 22, zygote. Fig. 23, longitudinal section of embryo sac showing zygote and first division of endosperm nucleus. Fig. 24, after small basal cell cut from primary endosperm cell. Fig. 25, free nuclear divisions in upper derivative from primary endosperm cell; basal apparatus still unicellular. Fig. 26, chalazal portion of endosperm showing multicellular basal apparatus. Fig. 27, degeneration of basal apparatus. Fig. 28, diagram of longitudinal section of mature seed. Fig. 29, portion of longitudinal section of mature seed showing seed coat and endosperm.

finally disappear. Nucellar cells persisting at the chalaza greatly elongate, while those next to the inner integument develop into a hypostase. This hypostase, a structure of universal occurrence among the Rosaceae and found in some of the Saxifragaceae, has become fully differentiated by the time the gametophyte has 8 nuclei.

Simultaneously with elongation of the megagametophyte further differentiation of the egg apparatus takes place. At the beaked extremities of the synergids are delicate longitudinal striations, the filiform apparatus (figs. 20, 21), occupying nearly half the length of the synergids. These structures stand out clearly when stained with safranin-light green. The filiform apparatus stains green while the distal cytoplasm of the synergids stains a deep red. This filiform apparatus exhibits a cellulose reaction similar to that found in *Parnassia* (19). The megagamete develops a big basal vacuole, and its nucleus comes to lie at its distal end (fig. 20).

The polar nuclei may remain together near the egg apparatus without undergoing actual fusion. Sometimes they can be found lying near the middle of the greatly elongated megagametophyte. They usually fuse before fertilization takes place.

A megagametophytic character conspicuous in *Lyonothamnus* is the chalazal elongation prior to fertilization. Among the Saxifragaceae such chalazal elongation is of rare occurrence. In some saxifragaceous genera (*Parnassia* 19, *Philadelphus* 7) there is more or less micropylar elongation of the gametophyte. As a rule the gametophyte of the Rosaceae shows a characteristic chalazal growth. In some of the rosaceous genera JACOBSSON-STIASNY (13) finds it to be either large and oval (*Spiraea*, *Sorbus*, *Rubus*, *Alchemilla*, *Rosa*), cylindrical (*Sanguisorba*, *Agrimonia*), or dumb-bell shaped (*Mespilus*, *Chaenomeles*, *Cydonia*, *Malus*, *Prunus*, *Cerasus*). While this chalazal enlargement found in *Lyonothamnus* is not universal among the Rosaceae, it is indicative of a rosaceous rather than of a saxifragaceous tendency.

SEED

Attempts were made to trace the course of the pollen tube from the stigma to the ovule with the use of resorcin blue dissolved in 50 per cent alcohol. Paraffin sections of pistils collected at hourly intervals up to 48 hours after opening of the flowers did not yield results.

Such preparations showed pollen tubes for a few millimeters down the center of the style and in the nucellus, but they were not recognizable in the intervening tissue. As soon as the pollen tube reaches the nucellus, it grows to the base of and between the two synergids. No actual discharge of the microgametes nor fertilization has been observed. As soon as a zygote is formed its nucleus takes a basal position (fig. 22). The synergids show signs of degeneration with their filiform apparatus still distinct, their distal cytoplasm staining deep red, and their nuclei not at all recognizable.

While GRAY (**8**) reported at least one or two mature seeds in each follicle he examined, I do not find this to be the case. One or two apparently plump seeds may be found within a single follicle, but a microscopic examination shows that they contain no embryo. This is a common occurrence among Rosaceae, at least in the apple (**17**), pear (**26**), plum (**10**), cherry (**27**), etc., where the self-fertilized embryo fails to develop. No artificial pollination has been made in this investigation.

The zygote does not divide until some time after fertilization, when endosperm formation is well under way. Soon after fertilization the primary endosperm nucleus divides into two (fig. 23), one of which migrates to the chalazal end and the other remains at the middle of the embryo sac. As soon as the chalazal endosperm nucleus reaches the distal end of the embryo sac, there is a cytokenesis which cuts off a small cell at the lower end (fig. 24). Subsequent development is that usually found in a primary endosperm cell. There follow repeated divisions of the nuclei in the larger daughter cell (fig. 25), and a free cell formation follows this multinucleate stage. Without undergoing free nuclear divisions the small basal cell begins to divide, about the time of free cell formation in the upper cell, and eventually gives rise to a small conical mass of parenchymatous cells with dense homogeneous cytoplasm and distinct nuclei (fig. 26). This basal apparatus persists for some time, but eventually disintegrates (fig. 27) as the seed approaches maturity.

The similarity between the basal apparatus of *Lyonothamnus* and that of *Tillandsia usneoides* (**1**) is striking. Differentiation of the basal apparatus in *Lyonothamnus* takes place long after free cell formation is well under way, while the reverse is true for *Tillandsia;*

for example, the basal apparatus is formed soon after the first division of the endosperm nucleus.

Among the Saxifragaceae cell divisions may follow the first division of the primary endosperm nucleus, or its primary endosperm cell may become multinucleate before cell division. Soon after fertilization, the endosperm nucleus in *Heuchera purpurea* (7) divides at the chalaza; one part migrates to the center of the embryo sac while the other remains at the chalaza. This binucleate cell becomes unequally divided into two daughter cells, each of which divides repeatedly. In *Chrysosplenium alternifolium* (7) we also have the division of the primary endosperm cell into two unequal cells, in both of which free nuclear division takes place. Cell formation takes place first in the lower cell, from which a basal apparatus of about 50 cells (persisting as a crushed tissue in the mature seed) is developed. The same is true of *Saxifraga granulata* (7), except that an 8-celled basal apparatus is developed. In *Francoa appendiculata* (7) the whole embryo sac becomes filled with free endosperm nuclei; this is followed by cell formation only at the upper portion of the sac, while the basal end which functions as a haustorium remains multinucleate. From these fragmentary data it appears that the Saxifragaceae are mostly characterized by a division of the primary endosperm cell into two daughter cells, the lower end of which by one method or another develops into a basal apparatus.

Development of the endosperm among the Rosaceae begins with free nuclear division, which is later followed by cell formation. JACOBSSON-STIASNY (13) reports that the endosperm entirely fills the embryo sac, and this is later reduced by the developing embryo. In *Prunus* RUEHLE (21) finds an interesting endosperm formation. In this plant free nuclear divisions of the endosperm nucleus occur, and at the time divisions are about complete, cell formation takes place at the upper portion of the embryo sac, while the basal end remains nuclear and functions as a haustorium. The endosperm of *Lyonothamnus* is decidedly saxifragaceous in character, in that a cellular basal apparatus, a structure unknown in the Rosaceae, is formed.

Because of the scarcity of developing embryos, no detailed account can be given of the embryonal development. At the time the

cotyledons are becoming differentiated, the embryo has a massive suspensor 2–3 cells broad and 3–6 cells long. The basal cell of the suspensor is not enlarged. JACOBSSON-STIASNY (**12**) finds that in *Saxifraga* and *Chrysosplenium* a suspensor haustorium is formed. She cites GOEBEL as finding a suspensor haustorium in Ribesiaceae. GÄUMANN (**7**) pictures an embryo of *Chrysosplenium alternifolium* with two basal cells of the suspensor greatly enlarged. Among the Rosaceae, PÉCHOUTRE (**20**) finds no indication of an enlargement of the basal suspensor cell, except in *Spiraea filipendula*. In *Geum urbanum*, SOUÈGES (**25**) as well as PÉCHOUTRE (**20**) shows a suspensor of a row of cells 5–6 layers high. From the data at hand the embryo of *Lyonothamnus* seems to be more rosaceous than saxifragaceous in nature.

The Saxifragaceae and Rosaceae can be differentiated in the morphological structure of their mature seed. In Saxifragaceae GÄUMANN (**7**) observed that the inner layer of the inner integument is sometimes cutinized (*Francoa*) or else thickened (*Heuchera*), while the outer integument develops characteristic outgrowths which give the seed a jagged surface. Among the Rosaceae, PÉCHOUTRE (**20**) finds that there is never any sclerification or notable thickening of the inner integument, and that this tissue takes no particular part in the protection of the seed. The outer integument (except *Pirus* and "Amygdalées") has a sclerification in its outer layer. Some or few of the subepidermal parenchymatous cells persist unmodified; the other hypodermal cells are crushed and form a membranous layer.

The mature seed of *Lyonothamnus* (fig. 28) possesses a coat derived from both inner and outer integuments of the ovule. The innermost portion (fig. 29) derived from the inner integument is not thickened and contains tanniferous material. The remaining cells of the inner integument become crushed. The superficial layer of the outer integument becomes highly sclerified, the layer immediately beneath being composed of cells with unthickened walls and protoplasm rich in tannin. The remaining cells of the outer integument become crushed. The endosperm in the mature seed is composed of 7–9 layers of cells at its thickest portion and is thinner at both ends. Within the endosperm is an embryo which consists of a

large radicle directed against the micropyle, and a pair of fleshy planoconvex straight cotyledons. This feature of the mature seed of *Lyonothamnus* is decidedly rosaceous rather than saxifragaceous in character.

Summary

1. In its floral morphology *Lyonothamnus* possesses both saxifragaceous and rosaceous features. Development of the carpels before all the stamens are formed agrees with some of the Rosaceae and a few of the Saxifragaceae. The one-celled archesporium, the failure to form a "coiffe épidermique," the absence of an obturator, and the development of a cellular basal apparatus are features purely saxifragaceous in nature. The differentiation of many nucellar cells which exhibit characters typical of spore mother cells indicates a close relationship with the Rosaceae. The peculiar elongation of the megagametophyte prior to fertilization, and its seed characters (simple modification of the integument, large embryo with a pair of fleshy planoconvex straight cotyledons, unenlarged basal suspensor cell, and sparingly developed endosperm) are features found among the Rosaceae.

2. While many of the floral characters of *Lyonothamnus* are either saxifragaceous or rosaceous in nature, the formation of two series of ovules in its ovary is a morphological criterion by which ENGLER and PRANTL (5) separate the Cunoniaceae from the Saxifragaceae. The family characteristics of Cunoniaceae and Saxifragaceae are so closely alike that early taxonomists considered Cunoniaceae a tribe of the Saxifragaceae. It seems evident that we are dealing here with a genus possessing heterogeneous morphological characters which tend to obliterate the distinction between Rosaceae and Saxifragaceae, and at the same time possessing a cunoniaceous arrangement of ovules.

3. It is unfortunate that no morphological studies of genera closely related to *Lyonothamnus* in both Rosaceae (*Vauquelinia*, *Lindleya*, *Photinia*) and Saxifragaceae (*Jamesia*, *Fendlera*), as well as those in the Cunoniaceae, have been made. Until such studies are made, *Lyonothamnus* should perhaps stand as a transitional form between Saxifragaceae and Rosaceae on the one hand, and Cunoniaceae on the other.

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THE LAYERING HABIT IN SITKA SPRUCE AND THE TWO WESTERN HEMLOCKS

WILLIAM S. COOPER

(WITH FOUR FIGURES)

In a paper published in 1911 (1), I discussed the habit of layering among conifers, especially as observed upon Isle Royale, Lake Superior. The scanty literature of the subject was also summarized, thirteen references, mostly European, being found. From the literature and my own work the following conclusions were reached. The habit of layering is common among coniferous trees, and particularly characteristic of the genera *Picea* and *Abies*. It occurs more frequently and attains more striking development with increasing latitude and altitude. Because of its effect upon the abundance and character of the reproduction, it is often an important factor in forest dynamics.

Since 1911 three further contributions have appeared. In 1913 FULLER (3) published a brief account of layering in the black spruce (*Picea mariana* (Mill.) BSP) as observed in the Saguenay region of Quebec. According to this report, the habit is characteristic of trees growing in open stand upon bare granite hills, each trunk being surrounded by a thick mat of basal branches, many of which root and become erect, so that the parent acquires a ring of daughter trees. Sometimes the original trunk dies and the result is a circular area covered with vigorous young upright shoots. The habit is of considerable importance in the establishment of forest upon bare granite areas.

In 1923, in a paper (2) dealing with forest development after glacier retreat at Glacier Bay, Alaska, I included a brief description of layering in the Sitka spruce (*P. sitchensis* Carr.). LUTZ, in 1930 (5), noted an instance of layering in the mountain hemlock (*Tsuga mertensiana* Carr.).

During a more recent visit to Glacier Bay, in the summer of 1929, many new facts came to light, so that a further report seems justified. It was discovered that the coast hemlock (*Tsuga heterophylla*

Sarg.) and *T. mertensiana* also layer, although the Sitka spruce does so most abundantly and vigorously.

The spruce, when growing in a pioneer environment, first develops a low bushy form, with the oldest branches lying on the ground. It is only such open-grown individuals that layer, since healthy basal branches are necessary. Rooting, however, will not occur until the prostrate branch becomes well covered by plant waste, and this must require several growing seasons at least. The branch may thus attain a considerable horizontal length before the change in geotropic response occurs. Adventitious roots are finally produced, the branch turns erect, radial symmetry is substituted for dorsiventral, and a treelike shoot is the result.

In areas transitional between willow thicket and forest, one finds apparent groups of young spruces which in reality are made up of a parent tree surrounded by layered shoots of almost equal height. Such a group is illustrated in my earlier paper (**2**, fig. 8). The branches which produce the daughter trees leave the main trunk at the surface of the ground or a little above it, or are completely buried by vegetable débris. The horizontal branch is slender close to the trunk, but becomes progressively thicker outward, reaching a maximum in the neighborhood of the upward bend (fig. 1). Under such open conditions, where there is an approximation to equality between parent and daughter shoots, any or all may form a part of the mature stand when the community becomes a closed forest. Where the parent trunks are more closely placed, the layering shoots, although originating as basal branches of open-grown trees, soon become overshadowed by the development of their parents, and persist for many years beneath the dense canopy in suppressed condition. Even these have their possibilities, however, for the destruction of one or more of the parent trees may provide an opportunity for vigorous renewal of growth.

Such an area was found on the east shore of Glacier Bay, just within the entrance (station 52). The appearance here was of a closed stand of submature spruce with frequent seedling trees of the same species beneath. It was soon discovered, however, that every apparent young tree was in reality a layered branch. A quadrat 10 m. square was laid out and a complete survey made (fig. 2). The

area was controlled by four spruces, ranging in diameter from 42 to 58 cm. and in age from 51 to 82 years. A single coast hemlock was present, 29 cm. in diameter and 58 years old. Three relict clumps of willow and one of alder, all low in vitality, still survived. The char-

FIG. 1.—Layered branch of *Picea sitchensis*, Strawberry Island, Glacier Bay, Alaska

acteristic ground cover of forest mosses was rather well developed, and the herbs, as usual, were few. The layering branches were found to leave the trunk below the present soil surface, and the horizontal portions, lying approximately on the surface of the mineral soil, were entirely concealed beneath several inches of litter. All the erect shoots showed the effects of suppression, but in differing degree. The average width of ring for the five dominant trees was 2.92 cm.; of the thirteen layering shoots, sectioned at the base of the erect por-

tion, 0.437 cm., with an extreme range of 0.205–1.141 cm. Suppression must be due, not to any direct dependence on the parent trunk, but to competition between parent and daughter shoots behaving as independent individuals.

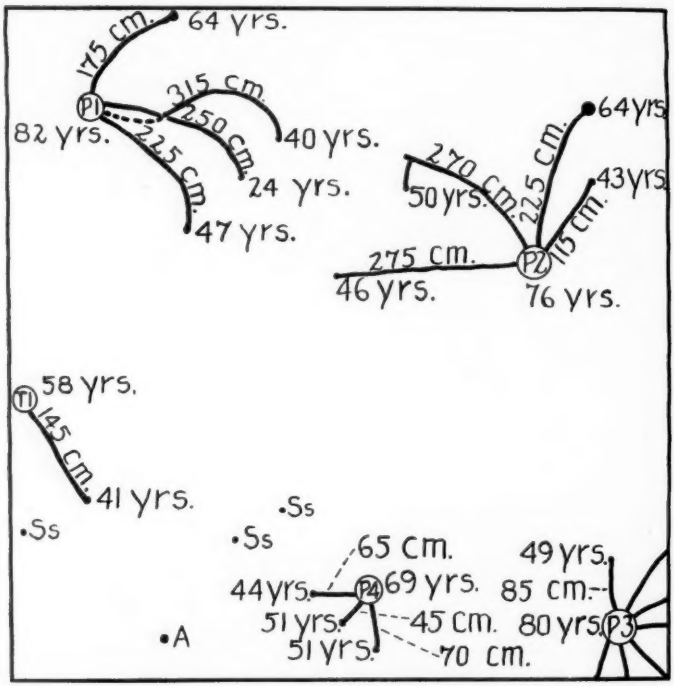

FIG. 2.—Quadrat in submature forest, east shore of Glacier Bay, Alaska, 10 m. square; parent trees and layering branches: *P = Picea sitchensis; Th = Tsuga heterophylla; A = Alnus tenuifolia; Ss = Salix sitchensis.*

Sections of the layering shoots show consistently a brief initial period of slow growth followed by a period of comparatively rapid increase, probably associated with the development of abundant adventitious roots before the forest canopy had become thoroughly closed. The final rings, belonging to the period of suppression, are almost microscopic. The length of the horizontal portion varied

from 45 to 315 cm. One erect shoot had developed from a secondary branch, the original terminal having been destroyed. Difference in age between parent and daughter shoot ranged from 8 to 58 years. Most of this is accounted for in the period of horizontal growth, but a portion is due to unavoidable uncertainty in counting because of closeness and incompleteness of rings. Undoubtedly these shoots have at least as good a chance of surviving and becoming members of the dominant stand as would seedlings under like conditions, and the layering habit is therefore of some consequence in the dynamics of the forest, here as on Isle Royale. The extreme likeness in appearance to seedling reproduction constitutes a warning against the drawing of too hasty conclusions in such a forest.

Two small layering branches, one from a spruce, the other from a coast hemlock, were brought from Glacier Bay and subjected to detailed analysis. The first was collected at station 22, in an area transitional between willow thicket and spruce forest, dense shade having not yet developed. The horizontal portion was 80 cm. long and the erect shoot 80 cm. high. Adventitious roots were present at intervals along the horizontal portion, mostly but not exclusively grouped in the vicinity of the annual bud scars. The diameter of the branch at the surface of the parent trunk was 7 mm., the cross-section being circular and continuing so as far as the third bud scar. At the second bud scar began a gradual increase in diameter, which continued to a point just short of the upward bend. Sudden increases in thickness were evident where the principal roots originated, and, in addition, local swelling also occurred at these points. Asymmetry began to show itself at the third bud scar, the vertical diameter increasing faster than the horizontal, this condition persisting through the region of the bend and disappearing in the erect portion. Maximum asymmetry occurred between the fourth scar and the bend, the average dimensions of three cross-sections being 13×17 mm.

This branch was sectioned for microscopic study at the surface of the trunk and just beyond each annual bud scar. The results are presented diagrammatically in fig. 3*A*, no attempt being made to indicate the varying thickness of the rings. It was possible to trace groups of rings and in many cases single ones from section to section, and thus to work out with considerable exactness the history of the

branch. Triangle *a-d-e* represents a central cone of wood (or rather one-half of it) made up of five wide rings in section 0, followed by four, three, two, and one in the succeeding sections. It is evident that in the first five years of its life (plus a probably brief period represented by the portion buried in the trunk) the branch grew rapidly, obtaining a length of 65 cm. During the next eight years (*d-e-c-b*) growth was slower, 43 cm. of length being added and a thickness of wood less than the total product of the previous five

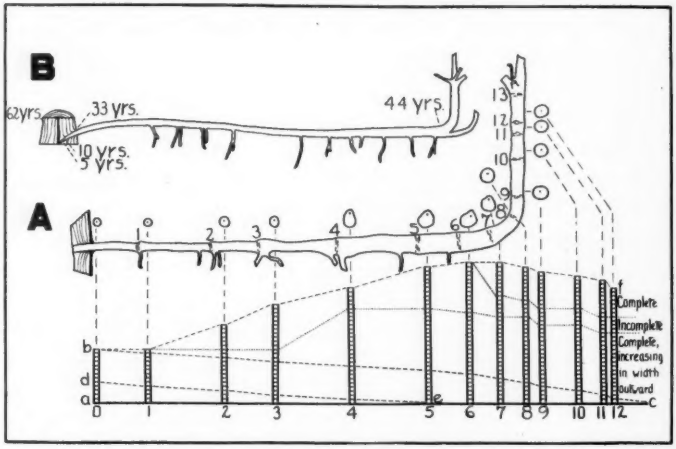

FIG. 3.—*A*, anatomy of layered branch of *Picea sitchensis*, Glacier Bay, Alaska; *B*, layered branch of *Tsuga mertensiana*, Glacier Bay.

years. After the thirteenth year growth near the trunk ceased, but new rings were added in the outer portion.

The triangle *b-c-f* indicates the wood increment after growth near the trunk had ceased. We have here the anomaly of an increase in the number of growth rings with increasing distance from the parent trunk. The evident cause is the acquisition of an independent absorbing apparatus in the form of adventitious roots. As to the location of the first-formed of these, with resulting first additional wood production, it is impossible to be certain. The largest roots were those near the third and fourth bud scars, and the most abrupt enlargement is associated with these. The sudden increase in the

new set of complete growth rings between sections 3 and 4 is a significant point. The outer half of the branch, too, because of its weight, would be likely to be first covered by litter. The lesser amounts of wood nearer to the trunk might well have been added later, as this portion finally became buried and produced roots.

The branch tip seems to have turned erect at about the eighth year. It is true, of course, that an independent change of growth direction does sometimes take place in a matured wood structure, but in the present case the evidence indicates that the change was accomplished by the growing tip itself within the space of a year or two. The initiation of eccentricity, and so doubtless the first production of adventitious roots, dates from the same year; and beyond the ninth scar there is no perceptible eccentricity, even in the earliest rings.

It is possible to date any ring in any section by adding the number of the ring in the section and the number of bud scars between it and the trunk, assuming the innermost ring of section 0 as number one, and assuming also, of course, continuity of growth. Using this method we find that pronounced eccentricity first became manifest between the eighth and thirteenth years in the life of the branch. Two periods are sharply distinguished in the time during which the adventitious root system functioned. The first was characterized by the production of complete but strongly asymmetric rings. During this period the annual wood increment gained in thickness from year to year almost without interruption. In the horizontal portion by far the greatest amount was added to the lower side; in the erect portion (sections 9–12) there was an equivalent increase in wood production, but with equal development along all radii.

In about the twenty-eighth year the period of rapid asymmetrical growth came to an end. It was followed by a time of exceedingly meager wood production, with complete failure on the upper side, resulting in a group of partial and very narrow rings. The suddenness of the change is emphasized by the fact that the first ring of this period appears in the sections as an arc of less than 150°. The second overlaps the first to some extent, and the succeeding rings in many cases closely approach completeness. In the horizontal portion the rings remain incomplete to the last, increasing somewhat in width,

then decreasing, at the same time becoming narrower in circumferential extent (fig. 4). In the upright portion, four incomplete rings are succeeded by a second set of wider complete ones. The sudden decrease in wood production must be attributed to the onset of unfavorable conditions in environment or in the plant itself. There is

FIG. 4.—Section of layered branch of *Picea sitchensis* (section 5 of *A*, fig. 3)

no clue as to its nature. Partial recovery brought about in the horizontal portion a radial widening of the incomplete rings; in the upright portion both widening and completion.

A small layering branch of mountain hemlock was also studied (fig. 3*B*), and in this it was possible to trace the branch to its origin at the center of the trunk. At two points within the trunk the branch showed five and ten rings; at the surface of the trunk 33, and just before the bend 44. The horizontal portion was about 75 cm. long and bore scattered roots from a point 30 cm. from the trunk as far

as the bend. The erect portion was 45 cm. tall. This branch showed the same features as the last: a circular cross-section near the trunk and an elliptical section near the bend, with most of the new wood added to the lower side. A very uncertain count of bud scars indicates a minimum age of 11 years to the point, just short of the bend, where 44 rings occur. A minimum age of 55 years is thus established for the age of the branch as a whole. The age of the trunk at the point of origin of the branch is 63 years. Eight years are thus unaccounted for, which may be due either to the impossibility of an accurate count or to the complete failure of wood formation at some time during the history.

It is worth while to attempt a physiological explanation of the phenomena displayed by these layering branches. Conditions incident to burial in vegetable débris cause the production of adventitious roots, principally upon the lower side. The use of foods in root building causes a general flow of these substances toward the roots, and a retarding or complete stoppage of flow back into the trunk. Practical cessation of growth in the proximal portion of the branch results. The adventitious roots pour water into the lower side of the horizontal branch. The water takes the line of least resistance and flows mainly through the lower part of the branch toward the growing tip. As a response to vigorous water flow and ample food supply, the production of wood cells should be most abundant on the lower side of the branch beyond the point of origin of the innermost important adventitious roots. The distal portion of the branch thus becomes eccentric, with the main body of wood below the growth center. It is true that in many cases gravity alone is sufficient to produce eccentricity in a horizontal organ. Experimental work now in progress shows this to be the case in the branches of Monterey cypress (*Cupressus macrocarpa*). In these spruces and hemlocks, however, the perfect circularity of the proximal portion of the branch shows that gravity alone is not the cause; the development of eccentricity is plainly connected with the production of adventitious roots.

As to the cause of the upward bend, an explanation suggests itself which is extremely plausible so far as the present cases are concerned: that the bending is due to faster growth on the lower side in the region of the tip where the tissues are still plastic. The point at

which it occurs is determined by the time when the flow from the newly formed adventitious roots first makes itself felt. When perpendicularity is attained the flow is no longer differential, the new position is made permanent by the fixation of tissues, and eccentricity gives way to perfect concentricity. Radial symmetry in the arrangement of the secondary branches is a final result. This explanation has the merit of extreme simplicity, and one is tempted to apply it to all the numerous cases in which lateral shoots, ordinarily plagiotropic, suddenly become orthotropic. GOEBEL (4) offers a somewhat similar but less definite theory, which is discussed in my former paper (1). GOEBEL thinks that a vigorous stream of water and other nutritive materials induce the orthotropic habit, and that the main shoot of a plant is therefore erect, while the less abundantly nourished laterals are plagiotropic. When the terminal shoot is removed the main stream is deflected into the uppermost lateral, which in consequence becomes erect. In the case of a layering branch the increased flow is due to the activity of the adventitious roots, the result being the same. GOEBEL'S explanation is rather vague in its mere assumption of a general stimulus; the suggestion I have offered at least provides a possible mechanism.

Unfortunately for both, there are a few cases which seem to range themselves in opposition. One, described in my former paper (1), was a balsam fir that grew in an open rock crevice. Several of the lower branches descended into the crevice a short distance, finally turning erect and developing a treelike form. No soil was present, no roots were produced, and the branch remained entirely dependent upon the parent trunk. In dense forest at Glacier Bay I found numerous seedling plants of mountain hemlock which took the form of prostrate mats, rooting abundantly but showing no tendency to turn erect.

The problem is of considerable physiological interest, and is evidently not a simple one. It would seem to be susceptible of experimental investigation, and Sitka spruce is recommended as promising material for such a study.

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ORIGIN AND DEVELOPMENT OF TISSUES IN STEM OF SELAGINELLA WILDENOVII

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 418

BERTRAM DONALD BARCLAY

(WITH NINETEEN FIGURES)

Introduction

Much work has been done on the anatomy of the mature structures in *Selaginella*, but the literature of the subject includes little information as to the exact origin of these tissues. The recent work of CHANG (2) on *Pteris aquilina*, and JOHNSON (4) on *Equisetum*, show many points at variance with the older accounts, especially as to what constitutes the exact limit between stele and cortex, and as to the origin of the endodermis and pericycle.

Selaginella was chosen as a subject of investigation for various reasons. It represents a group of plants generally possessing a single apical cell, which facilitates tracing the tissues to their exact origin. It belongs to a division of pteridophytes that has not been critically investigated for many years, while members of the Filicales and the Equisetales have only recently been reworked. Also the existing accounts of tissue origin and development in *Selaginella* do not agree, indicating the need of further work. Of the available species, *Selaginella wildenovii* Bak. was found to be the most favorable for this study. The young primary branches grow to a greater length without forking than in other species available. The individual cells are also larger than in other species.

This investigation deals first with the general gross and microscopic anatomy of the young primary stem, then with the apical cell and its derivatives, the origin and development of cortex, endodermis, and air spaces, pericycle, xylem, and phloem. The origin and development of the xylem and phloem are discussed only briefly at this time.

Material and methods

Collections were made at the University greenhouses during the winter months, since at this time of year *Selaginella wildenovii*

makes its most vigorous vegetative growth. The best fixation was obtained by the use of a formalin-acetic-alcohol mixture. The safranin-light green combination was found to be the most successful anatomical stain.

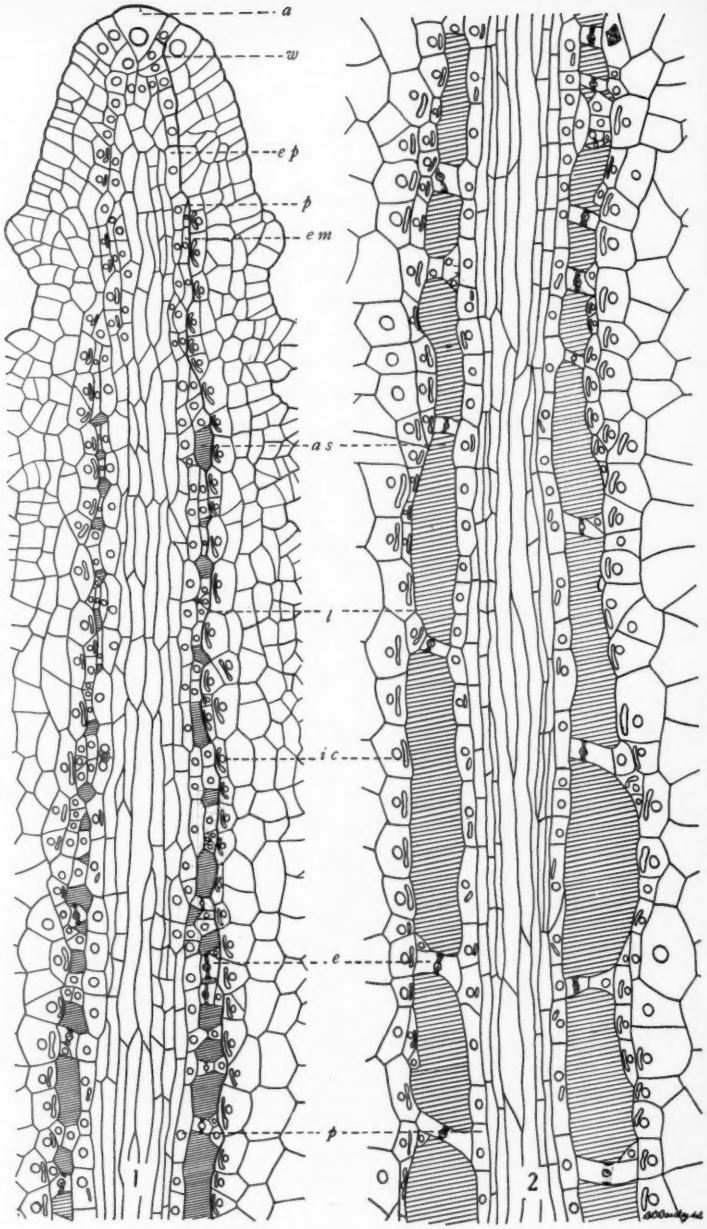
Investigation

General anatomy

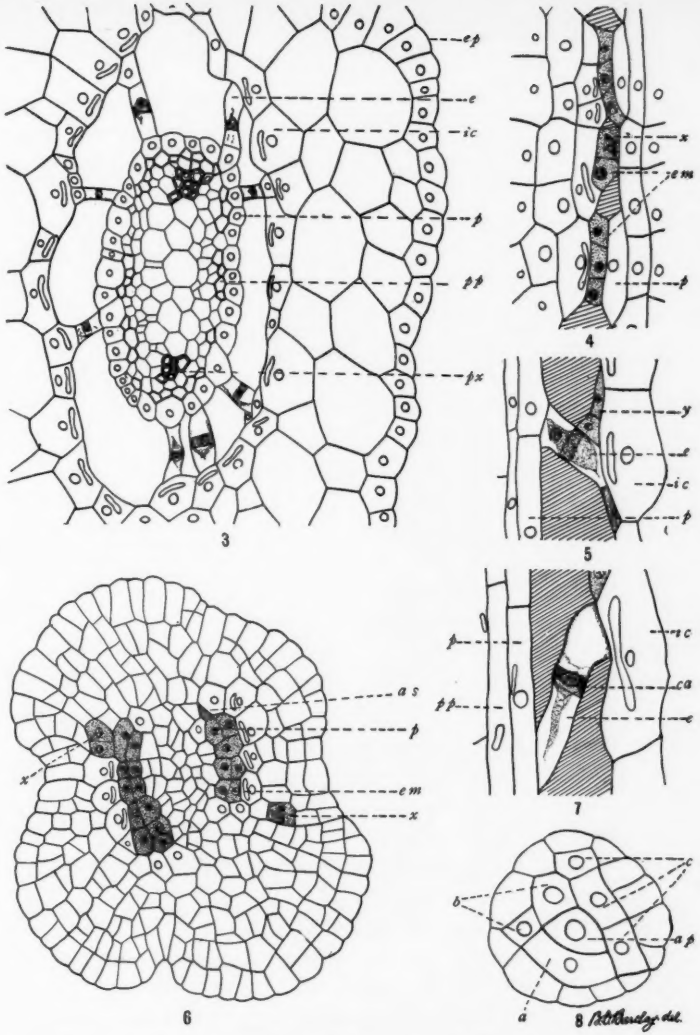
The stem of *Selaginella wildenovii* is flattened dorsiventrally. Two rows of small leaves are borne on the dorsal surface, and two rows of larger leaves on the ventral surface. A view of the dorsal surface of a young primary shoot shows that the young growing tip always bends upward, the whole stem curving slightly in this direction. The position of the growing point and the upward curvature of the stem make impossible a median longitudinal section parallel to the dorsiventral plane, and make difficult a cross-section through the apical cell. The median longitudinal section figured is cut at right angles to the dorsiventral surface.

In a well developed primary branch, the vascular cylinder consists of a single protostele, flattened dorsiventrally, with two lateral protoxylem points (fig. 3). The metaxylem develops centripetally and meets in the center of the stele. The xylem is entirely surrounded by one or two layers of protophloem, easily recognized by the unusually bright green color of the walls. The central cylinder is surrounded by the pericycle, generally one layer of cells in thickness. The cells of the pericycle contain a single chloroplast. Between the pericycle and the inner cortex is a broad air space, bridged by the elongated endodermal cells or by an endodermal cell united with one or more elongated cells without endodermal thickenings. These cells have a common origin with the endodermal cell, but unlike it do not develop Casparian thickenings, and may possess chloroplasts. The cells composing the inner layer of the cortex contain a single large chloroplast, U-shaped in cross and longitudinal section, and next to the cell wall that abuts the air space. All the other cortical cells contain chloroplasts, but these are smaller and different in form from those in the cells of the inner cortex.

Apical cell and its derivatives.—The stem grows by means of a tetrahedral apical cell. A transverse section of this apical cell



FIGS. 1, 2.—Median longitudinal section of young shoot at right angles to dorsiventral surface: *a*, apical cell; *w*, periclinal wall dividing first segment into stelar and cortical segments; *ep*, cell giving rise to endodermis and pericycle; *p*, pericycle; *em*, endodermis mother cell; *e*, endodermal cell; *as*, air space; *ic*, inner cortex; *l*, limit between stele and cortex; ×420.



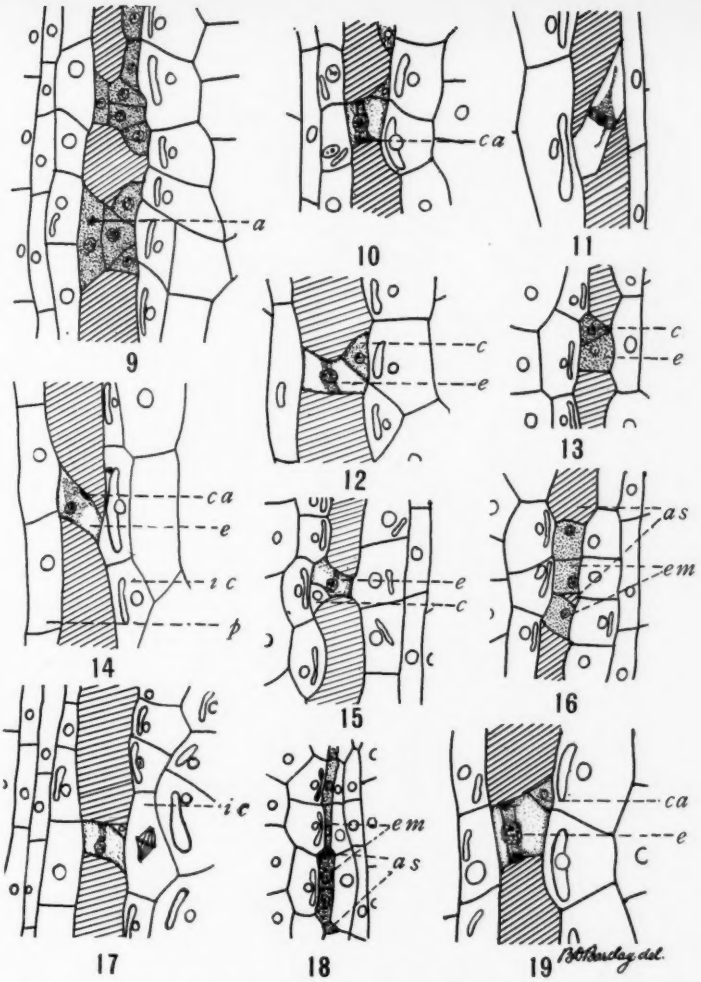
FIGS. 3–8.—Fig. 3, transverse section of young shoot, showing epidermis (*ep*), inner cortex (*ic*), endodermal cells (*e*), pericycle (*p*), protophloem (*pp*), protoxylem (*px*); ×570. Fig. 4, longitudinal section showing inner cortex to left of endodermis mother cells; endodermal mother cell (*x*) dividing by oblique wall (fig. 13) shows stage immediately following oblique division); ×655. Fig. 5, longitudinal section showing endodermal cell (*e*); stippled cells (*y*) are derivatives of endodermal mother cells; shaded areas indicate air spaces; ×570. Fig. 6, transverse section through young shoot at level at which endodermis and pericycle are first differentiated; ×570. Fig. 7, simple endodermal cell (*e*) stretched obliquely by differential longitudinal growth of central and cortical cylinder; *ca*, Casparian strip; ×655. Fig. 8, transverse section of apical cell and segments; *a*, first segment; *b*, second segment; *c*, third segment; cells composing second segment distorted by growth; ×655.

is shown in fig. 8. Seen in longitudinal section (fig. 1), the first segment of the apical cell divides by means of anticlinal and periclinal walls. The desmogen strand arises from the products of the inner half of the periclinal division, while the products of the outer half remain parenchymatous. The desmogen strand can be distinguished from the rest of the tissue five or six divisions below the apical cell (fig. 1).

CORTEX.—The outer cell resulting from the first division of the first segment of the apical cell forms by tangential and transverse divisions a cortical tissue four or five cells in thickness. The cortical cylinder increases in size by radial divisions of its cells (fig. 6), but the number of layers of cells does not increase for a long time. This increase of internal diameter of the cortical cylinder, together with its growth in length, brings about the formation of air cavities and the separation and stretching of the endodermal cells, as will be shown later.

ENDODERMIS AND AIR SPACES.—The endodermal cells can first clearly be recognized about 0.3 mm. from the stem tip by the Casparian strips which line the radial and end walls of the endodermal cell (fig. 1 *e*). In sections stained with safranin and light green, the Casparian strips are seen first as reddish points on the radial and cross walls, to which the cytoplasm adheres when the cells are plasmolyzed (fig. 19). The nucleus of the endodermal cell at this stage always lies in the plane of the thickenings (figs. 10, 19). An endodermal cell can be recognized several cells above the place where the Casparian thickenings first appear, by the manner in which plasmolysis occurs (fig. 15). The endodermal cell may be united directly to the pericycle on one side and to the inner cortex on the other (figs. 7, 11, 14). It may also be united directly to the pericycle on the inner side, and joined to the inner cortex by means of one or more cells which arose from a single endodermis mother cell.

The cells of the inner cortex with their conspicuous chloroplasts aid in tracing the inner limit of the cortex (fig. 1 *ic*). The inner layer of the cortex can thus be traced to within five cell divisions of the apical cell. From this point on it is an easy matter to trace the cell which will give rise to the endodermis (endodermis mother cell), to its common origin with the cell which will give rise to the peri-



FIGS. 9–19.—Fig. 9, longitudinal section showing derivatives of endodermal mother cells. Cell (*a*) will become endodermal (this cell lies next to pericycle); ×810. Fig. 10, endodermal cell shortly after appearance of Casparian strip; ×810. Fig. 11, obliquely stretched endodermal cell similar to that in fig. 7; ×550. Fig. 12, longitudinal section showing result of oblique division of endodermis mother cell shown in fig. 4; *e*, endodermal cell; *c*, sister cell. Fig. 13, similar to fig. 12, but showing earlier stage following oblique division of endodermis mother cell. Fig. 14, endodermal cell similar to that in fig. 7. Fig. 15, cells formed by oblique division of endodermis mother cell, similar to fig. 12. Fig. 16, longitudinal section showing formation of air spaces between endodermis mother cells. Fig. 17, longitudinal section showing elongation of inner cortex by transverse division. Fig. 18, longitudinal section showing formation of air spaces between endodermis mother cells; pericycle to right of endodermis mother cells, inner cortex to left. Fig. 19, longitudinal section through endodermal cell, showing first appearance of Casparian strip; figs. 12–19, ×810.

cycle. The mother cell of the endodermis and pericycle can be traced directly to the inner cell resulting from the periclinal division of the first segment of the apical cell. This inner segment gives rise to the initial cell of the endodermis and pericycle and the initial cell of the xylem and phloem.

The cells which later give rise to endodermis, at first form a compact tissue. Soon radial divisions occur in the cells of the cortex (fig. 6), and the whole cortical cylinder increases in diameter. Longitudinal growth of the cortical cylinder occurs at the same time. The desmogen strand, together with the pericycle, increases in diameter, but more slowly than the cortical cylinder. The increase in length of the part of the stele inclosed within the pericycle keeps approximate pace with the elongation of the cortical cylinder. The rate of growth of the endodermis mother cells does not keep up with the differential increase in diameter and length of the cortical cylinder and central bundle. As a result, the endodermis mother cells become stretched radially between the pericycle and inner cortex, and their radial walls become separated in the vertical and horizontal planes (figs. 1, 4, 6, 16, 18). An air cavity is thus formed between the pericycle and inner cortex, bridged by the endodermis mother cells. The endodermis mother cell may give rise directly to a single trabecula with its endodermal thickenings (figs. 7, 11, 14), uniting the pericycle to the inner cortex. The endodermis mother cell may also divide longitudinally and give rise to a trabecula composed of a single cell with endodermal thickenings next to the pericycle, and one or more cells uniting it to the inner cortex (fig. 9). An oblique wall may come in at the first division of the endodermis mother cell (fig. 4). In this case the daughter cell that has the firmest attachment to both pericycle and inner cortex stretches and becomes endodermal, while the other daughter cell is pulled away from the pericycle and lies next to the cortex (figs. 5, 10, 12, 13, 15). Occasionally a group of endodermis mother cells may become separated from the pericycle at an early stage and remain as a thin layer of cells against the inner cortex (fig. 9). This is the "trabecular cortex" of VLADESCU (9). Chloroplasts were not observed in cells with endodermal thickenings, but were found in other trabecular cells.

PERICYCLE.—The cells of the pericycle are at first of the same dimensions as the cells of the neighboring desmogen strands and the endodermis mother cells. The desmogen cells divide vertically and become much narrower than the pericyclic cells (fig. 1). The endodermis mother cells do not increase in length, and become stretched and separated. By this time the pericycle forms an easily distinguishable tissue. The pericyclic cells contain a single flattened chloroplast, generally located against the cell wall away from the air space.

XYLEM AND PHLOEM.—The first protoxylem to appear can be recognized in the leaf traces. The first protoxylem elements in the stem make their appearance a short distance below the first lignification in the leaf trace. This occurs about 0.3 mm. below the stem tip. The first protophloem appears about 0.05 mm. below the first protoxylem. Lateral sieve plates were observed in the protophloem sieve tubes. Metaxylem and metaphloem are differentiated much later.

Discussion

Various types of apical cells have been reported in the Selaginellaceae. PFEFFER (5) found that the stem of *Selaginella martensii* grows by means of a 2-sided apical cell. TREUB (7), working on the same species, reported that growth took place by means of either a 2-sided, 3-sided or 4-sided apical cell. HOFMEISTER (3) found a 2-sided apical cell in *S. hortensis* and *S. galeotii*. The writer finds that *S. wildenovii* possesses a 3-sided apical cell. BRUCHMANN (1), in his studies on *S. spinulosa*, found apical growth taking place by means of a general meristem, as in the Lycopodiaceae. STRASBURGER (6) reported that *S. wallichii* possesses a group of two initial cells. This seems to show that there is in *Selaginella* every intergradation, from a single apical cell to a general meristematic group. The different kinds of apical cell reported, and the variance of opinion on the type of apical cell possessed by one species, indicate that the apical situation in the genus as a whole is in need of careful reinvestigation.

VAN TIEGHEM (8) found that in *Selaginella inaequifolia*, *S. wallichii*, and others, each segment of the apical cell divides by means

of a tangential wall, then by a second periclinal division exterior to the first. Of the three segments thus formed, the inner one gives rise to the central cylinder and pericycle. The middle segment forms the endodermis and inner cortex, and the outer segment produces the outer cortex and epidermis. He reasons from this that the endodermis forms the innermost layer of the cortex and the pericycle the outermost layer of the stele. He adds that this situation is general for the ferns and specific for *Marsilea*, *Selaginella*, and *Equisetum*. Although he gives an authoritative account, no figures showing his findings in the stem are presented.

VLADESCU reports a different situation in *Selaginella*. His report describes in detail the origin of tissues, but he gives no figures and does not mention the species with which he worked. According to his findings, the first segment of the apical cell (form not given) divides into three segments, as reported by VAN TIEGHEM. The outermost segment forms the epidermis and the outer cortex. The middle segment forms the pericycle, endodermis and its derivatives, and the inner cortex, while the innermost segment gives rise to the vascular tissues. He finds a common origin for endodermis, pericycle, and inner cortex, and reports that the endodermis and pericycle arise from a single mother cell. His conclusion is that the line between stele and cortex does not divide the endodermis from the pericycle, but that the stele includes the endodermis and inner cortex. STRASBURGER agrees with VLADESCU'S findings, and adds that he had previously arrived at the same conclusions in his work on *Selaginella* and *Lycopodium*.

In *Selaginella wildenovii*, which possesses a tetrahedral apical cell, the first segments of the apical cell divide tangentially to form only two segments. The outermost gives rise to cortex only, and the innermost forms the vascular elements, pericycle, and endodermis with its derivatives. The limit of stele and cortex in this species is thus between the endodermis and the inner cortex. This situation corresponds exactly with the findings of CHANG (2) in *Pteris aquilina*.

In view of these conflicting reports, it is evident that much work on the origin and development of tissues in this genus is still needed.

Summary

1. The stem of *Selaginella wildenovii* grows by means of a tetrahedral apical cell.

2. The endodermis, pericycle, and vascular strand have a common origin and are all stelar. The endodermis forms the outermost layer of the stele.

3. The endodermis and pericycle arise from a common initial.

4. Elongation of the endodermal cells and formation of air spaces are explained by the mechanics of differential cell growth.

5. The protoxylem differentiates first in the leaf traces and later in the stem. The protophloem differentiates slightly later than the protoxylem.

The writer wishes to express his indebtedness and gratitude to Professor W. J. G. LAND, at whose suggestion and under whose direction this study has been made.

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BRIEFER ARTICLES

A HYBRID LOBELIA

Reference is made in the manuals to hybrids of *Lobelia syphilitica* L. and *L. cardinalis* L., but not to hybrids of the first species with *L. puberula* Michx. Plants of *L. syphilitica* and *L. cardinalis* were grown for many years in the wild flower garden, with no instance of crossing. The more recent introduction of *L. puberula* into the garden has resulted in the appearance of a number of forms of blue lobelia which can be explained only as hybrids. These hybrid plants (*Lobelia syphilitica* L. × *puberula* Michx.) are densely and finely puberulent throughout, and the larger anthers are minutely bearded, characters of the *puberula* parent. In stoutness and leafiness of stem, the plants resemble *L. syphilitica*. The flowers are nearly the size of those of *L. syphilitica*, with the stout tube and strongly corrugated lower lip. The calyx resembles that of *L. syphilitica* much more than it does *L. puberula*, the size of the strongly deflexed auricles varying somewhat on different plants. Leaf shape is intermediate between the leaf shapes of the two parents, the apex being more like that of *puberula*.

While this hybrid form has not been observed in the field, it may be expected where the two species occur together. From a distance it might be mistaken for a plant of *L. syphilitica*, but on feeling the leaves, the touch would at once suggest *L. puberula*.

Among the segregates of the *Lobelia syphilitica* × *puberula* hybrid a considerable range of forms is evident. Plants vary in respect to some characters, as leaf thickness and obtuseness of apex, form of calyx tube, size and shape of auricles, flower color, and form of lower lip. This last, however, in every case shows the divergent sides evident in fresh specimens of *L. puberula*, in contrast to the parallel or even slightly convergent sides of the lower lip of *L. syphilitica*. Characters of the *puberula* parent which appear to be dominant, prevailing in all forms although varying slightly in intensity, are puberulence of leaves and stem, puberulence of anthers, and one-sided form of inflorescence. The arching stamen tube of *L. syphilitica* does not appear in any of the forms observed.

While most of the segregates appear to be somewhat intermediate, although more strongly resembling *L. puberula*, one departs markedly in

flower aspect from the others. The flowers are larger than in either parent (about 3 cm. long); the lobes of the lower lip are broad and widely spreading; the inflorescence is compact (as in *syphilitica*) but tending to secund (as in *puberula*); the anthers are puberulent only on the angles; the auricles of the calyx are large and the calyx tube hemispherical as in *L. syphilitica*. This form surpasses in beauty either of the parents.

As the observations here recorded are based on only chance occurrence of plants, it is of course possible that some more recent forms are the result of second crossing and are not true F_2 segregates.

Such a variety of forms is at least possible, however, and it may be of interest to taxonomists that the combination of characters in at least one of them is such that it could not be ascribed to any species by the use of keys and manuals.—E. Lucy Braun, *University of Cincinnati, Cincinnati, Ohio.*

CURRENT LITERATURE

BOOK REVIEWS

Chemical Plant Physiology

Botanists, and especially plant chemists, will welcome LYON'S translation[1] of KOSTYCHEW'S *Chemische Physiologie*. The emphasis on chemical plant physiology is one of the recent important developments in plant physiology. As brought out by the author, this has been made possible by late contributions in physical and biological chemistry. The result is that modern textbooks of plant physiology present a different aspect from those published a few decades ago. Much more attention is given to the chemistry of plant processes and less to various phases of growth and movement, the physics and chemistry of which are very poorly understood. As stated by the translator, the German volume was published in 1926 as Part I of the author's *Lehrbuch der Pflanzenphysiologie*, the implication being that there would be a second volume dealing with the physical phases of plant physiology. The English edition has been brought up to date by the author's revisions, made late in 1929.

The book is divided into an introduction and eight chapters. In chapter I there is a discussion of those subjects that serve as a basis for chemical plant physiology: colloids, catalysis, enzyme action, velocity of chemical reactions, hydrogen-ion concentration, and related subjects. Chapters II–IV are devoted respectively to photosynthesis, chemosynthesis and nitrogen fixation, and the nutrition of plants by means of prepared organic compounds. Mineral nutrition is considered in chapter V, the metabolism of carbohydrates and proteins in chapter VI, and the fats, lecithins, organic acids, tannins, etc., in chapter VII. The last chapter is devoted to respiration and fermentation.

Taken as a whole, the book is a good summary of the life processes of the plant, considered in their chemical aspects. The researches summarized are mainly German and Russian, including, however, a number of the more important English contributions and some American ones. The translator has, in a degree, made up the lack of adequate references to American work. The book serves as a good source of information concerning KOSTYCHEW'S own researches. While he has in his investigation emphasized respiration, one is impressed by the breadth of his research interests. There is hardly a phase of plant physiology he has not enriched by original contributions. At the close of many of the sections there are considered the research methods used in that particular phase of the subject. These are considered mainly from the standpoint of the principle involved, although references are given from which the details of the methods may be obtained.—S. V. EATON.

[1] KOSTYCHEW, S., Chemical plant physiology. Translated and edited by C. J. LYON. pp. XV+497. *figs.* 45. P. Blakiston and Son. Philadelphia. 1931.

Ferns and flowering plants of Hawaii National Park

It is now more than 40 years since HILLEBRAND'S *Flora of the Hawaiian Islands* appeared. Meanwhile great advances have been made in the study of the Hawaiian plants, and hundreds of new species have been described. Such workers as C. N. FORBES, J. F. ROCK, and OTTO DEGENER have pushed forward their taxonomic studies with noteworthy zeal. Unfortunately, FORBES is now dead and ROCK has withdrawn to another field. It is all the more gratifying, therefore, to know that DEGENER remains to continue the work. His *Flora Hawaiiensis*, which he has in preparation, bids fair to be a really monumental work in plant taxonomy. His recently issued volume[2] is more popular in its treatments than a conventional flora is apt to be, yet even this smaller work has such a valuable storehouse of taxonomic information that it will prove indispensable to students of Hawaiian plants for years to come. A comprehensive glossary of native works is given. Much of the early history of Hawaiian exploration and discovery has been incorporated. There are various excellent photographs, and these are supplemented with 95 full-page plates, most of them original and of a high degree of excellence. In this way a not inconsiderable number of obscure Hawaiian species have received published illustration for the first time. Various new names which have been found necessary under nomenclatural rules have been omitted, the author preferring to leave such names for publication in his forthcoming *Flora Hawaiiensis*.

Aside from taxonomy, the volume makes a distinct appeal to many people of diverse tastes. The geology of the Hawaiian Islands, the ethnographic relations of the inhabitants, the wars of conquest and defense fought in bygone days, the tribal and family customs which once held sway, the primitive methods of preparing food—these and numerous other subjects receive a treatment that is both authoritative and absorbingly interesting.—E. E. SHERFF.

Physical Properties of Soil

An excellent summary[3] of our knowledge of the physical properties of the soil has been written by KEEN. It is one of the Rothamsted monographs on agricultural science, and meets the need for a book of this kind. As the author points out, it is only in recent years that the subject has been put on a firm scientific basis, yet the treatment of this field in current textbooks of agriculture is based largely on early ideas, many of which recent investigations have shown are erroneous. These investigations are well summarized in the book.

The volume opens with a historical introduction, in which there is treated the relations between the development of our knowledge of the physical properties

[2] DEGENER, OTTO, Illustrated guide to the more common or noteworthy ferns and flowering plants of Hawaii National Park. pp. xv+313. *Frontispiece and pls. 95, figs. 45.* Honolulu Star-Bulletin, Ltd. Honolulu. 1930.

[3] KEEN, B. A., The physical properties of the soil. pp. vi+380. *figs. 93.* Longmans Green Co., New York City. 1931.

of the soil and the development of cultivation implements. Chapter II has to do with the technical analysis of the soil. Chapter III considers the physics controlling the movement of water in the soil. The erroneous nature of the "capillary tube" hypothesis is shown. There follow chapters on soil properties in low moisture conditions, soil and clay pastes, the properties of soil and clay suspensions, soil constants and equilibrium points, physical properties of soil under various conditions, and cultivation and cultivation implements. The last two chapters have to do with factors controlling the temperature and aeration of the soil. The literature is critically summarized, and is presented in a clear, well organized, convincing manner. In common with the other Rothamsted monographs, there is a copious citation of literature. The general bibliography at the end of the book contains 263 titles. In addition there are numerous footnotes throughout the book giving other references.—S. V. EATON.

Elements of plant science

In recent years there have appeared numerous textbooks of botany for use in secondary schools and colleges, all with much similarity of contents. The volume by CHAMBERLAIN[4] is notable because it is unique in several ways. The text, carefully planned, is such a simple, clear, and orderly presentation of the most up-to-date botanical knowledge that even beginners in the subject can easily comprehend it. This clarity of statement and careful selection of material are of course possible because of the vast store of botanical knowledge possessed by the author, combined with more than 40 years of sympathetic contact with students of botany. All of the illustrations are original, have been specially made to fit the text, and are very accurate.

Part I, which can be used by schools without microscopes, takes up the structures in a natural manner: leaf, stem, root, flower, fruit, and seed; followed by a chapter on various important topics, and one on laboratory studies. Part II, devoted to the structure and development of plants, is a presentation of their orderly development, and is illustrated with a wealth of excellent drawings and diagrams accurately fitted to the text. The chapter on laboratory methods is a fitting close to what the reviewer believes is the most admirable textbook of botany he has ever seen for use in secondary schools and colleges.—W. J. G. LAND.

[4] CHAMBERLAIN, C. J., Elements of plant science. pp. xii+394. *figs. 321*. McGraw-Hill Book Co. New York. 1930.

GENERAL INDEX

Classified entries will be found under Contributors and Reviews. New names and and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

A

Abderhalden, E., work of 222
Adriance, G. W. 144
Antheridial dehiscence in Polypodiaceae 252
Arceuthobium, floral morphology of 42
Arnold, C. A. 77
Ascent of sap 395
Aspergillus, effect of metals on growth of 183

B

Babcock, E. B., work of 110
Barclay, B. D. 452
Beger, H., work of 222
Bidens, **mildbraedii** 308; **rhodesiana** 309; **setigeroides** 310; **skottsbergii** 311
Blakeslee, A. F., work of 111
Bluff succession along Missouri River 295
Bouteloua **pectinata** 103
Braun, E. Lucy 462
Brieger, F., "Selbststerilität und Kreuzungssterilität" 334

C

Cain, S. A. 22
Calfee, R. K. 183
Carya, foliar organs of 277
Castle, E. S. 206
Chamberlain, C. J. 220; "Elements of plant science" 466
Chemical plant physiology 464
Chlorophyll content and photosynthesis 223
Citrus cuttings, solubility changes of inorganic constituents of 213
Cladonia 336
Climate and plant species 336
Clipping plant, and effect on nodule development in soy bean 115
Collins, L. J., work of 110
Compositae, new 308
Contributors: Adriance, G. W. 144; Arnold, C. A. 77; Barclay, B. D. 452; Braun, E. Lucy 462; Cain, S. A. 22; Castle, E. S. 206; Calfee, R. K. 183; Chamberlain, C. J. 220; Cooper, W. S 441; Costello, D. F. 295; Coulter, M.C. 110; Cross, G. L. 65; Davies, P. A. 320; Dowding, E. Silver 42; Dustman, R. B. 395; Eaton, S. V. 115, 223, 333, 464, 465; Edgecombe, A. E. 1; Erlanson, Eileen W. 55; Erwin, A. T. 105; Featherly, H. I. 103; Frost, F. H. 83; Fuller, G. D. 222, 335; Guard, A. T. 97; Haas, A. R. C. 213; Halma, F. F. 213; Hartman, M. Elizabeth 252; Husted, L. 219; Isbell, C. L. 411; Juliano, J. B. 426; Lampe, Lois 337; Land, W. J. G. 466; Langdon, LaDema M. 277; Link, G. K. K. 109; Loehwing, W. F. 332; McHargue, J. S. 183; Michaels, W. H. 167; Montgomery, C. E. 225; Noé, A. C. 109, 222, 223; Ramaley, F. 336; Sherff, E. E. 308, 465; Shull, C. A. 334, 395; Smith, Cornelia M. 377; Smith, Fanny 395; Vokes, Margaret M. 194
Cooper, W. S. 441
Coprinus, sterigmata in 194
Cordaitean wood from Pennsylvanian 77
Costello, D. F. 295
Coulter, J. M., "A textbook of botany: revision" 332
Coulter, M. C. 110
Cross, G. L. 65
Cucurbits, nativity of 105

D

Davies, P. A. 320
Day-length, effect on nodule development of soy bean 115
Degener, O., "Floral guide to Hawaii National Park" 465

Dehiscence, antheridial 252
Dionaea, germination of seed 377
Dispersal of plants 335
Dobzhansky, T. L., work of 111
Dowding, E. Silver 42
Dustman, R. B. 395

E

Eaton, S. V. 115, 223, 333, 464, 465
Edgecombe, A. E. 1
Elements of plant science 466
Emerson, R., work of 223
Endosperm of maize 337
Erlanson, Eileen W. 55
Erwin, A. T. 105
Europe, flowering plants of central 222
Evans, A. W., work of 336

F

Featherly, H. I. 103
Fitting, H., "Strasburger's Textbook of botany" 220
Frost, F. H. 88
Fuller, G. D. 222, 335

G

Gager, C. S., work of 111
Germination of seed of Dionaea 377
Gold Coast, plants of 335
Goodspeed; T. H., work of 111
Grama grass, new species of 103
Great Smoky Mountains, vegetation of 22
Guard, A. T. 97

H

Haas, A. R. C. 213
Halma, F. F. 213
Hanson, F. B., work of 111
Harris, B. B., work of 111
Hartman, M. Elizabeth 252
Harvey, R B., "Plant physiological chemistry" 333
Hawaii National Park, flora of 465
Heys, Florence, work of 111
Husted, L. 219
Hybrid sterility and self-sterility 334

I

Immunological relationship of wheats resistant and susceptible to Puccinia 1
Ipomoea, regeneration in 411
Irvine, F. R., "Plants of the Gold Coast" 335
Isbell, C. L. 411
Isostigma **molfinianum** 312

J

Juliano, J. B. 426

K

Keen, B. A., "The physical properties of the soil" 465
Kiesel, A., "Chemie des Prototoplasmas" 223
Kostychew, S., "Chemical plant physiology" 464

L

Lampe, Lois 337
Land, W. J. G. 466
Lang, W. H., "Translation of the Strasburger Textbook of botany" 220
Langdon, LaDema M. 277
Layering habit in spruce and hemlock 441
Light-sensitive system in Phycomyces 206
Link, G. K. K. 109
Lobelia, a hybrid 462
Loehwing, W. F. 332
Lüdi, W., work of 222
Lyonothamnus floribundus 426

M

Maize, endosperm of 337
McHargue, J. S. 183
Meristem in Osmunda 65
Methods in plant ecology 222
Michaels, W. H. 167
Microchemical study of maize endosperm 337
Missouri River, bluff succession of 295
Montgomery, C. E. 225
Mosses, ecology of 225
Muller, H. J., work of 110
Mutations, X-ray 110

N

Nitrogen in regeneration of willow 320
Nodule development of soy bean 115
Noé, A. C. 109, 222, 223

O

Oliver, C. P., work of 110
Osmunda, meristem in 65

P

Painter, T. S., work of 111
Patterson, J. T., work of 110
Pecan, fruit-setting in 144
Penicillia 109
Pennsylvanian, cordaitean wood from 77
Permian flora, a new 109
Photosynthesis and chlorophyll content 223
Phototropic "indifference" and light-sensitive system of Phycomyces 206
Phycomyces, light-sensitive system of 206
Physiological chemistry 333
Polypodiaceae, antheridial dehiscence in 252
Preparation of copy 327
Puccinia, and immunological relationship of wheats 1

R

Ramaley, F. 336
Regeneration, in Ipomoea 411; in willow 320
Respiration of shoot and temperature changes 167
Reviews: Brieger's "Selbststerilität und Kreuzungssterilität" 334; Chamberlain's "Elements of plant science" 466; Coulter's "Textbook of botany: revision" 332; Degener's "Floral guide to Hawaii National Park" 465; Fitting's "Strasburger's Textbook of botany" 220; Harvey's "Plant physiological chemistry" 333; Irvine's "Plants of the Gold Coast" 335; Keen's "The physical properties of the soil" 465; Kiesel's "Chemie des Protoplasmas" 223; Kostychew's "Chemical plant physiology" 464; Lang's "Translation of the Strasburger Textbook of botany" 220; Ridley's "Dispersal of plants" 335; Thom's "The Penicillia" 109; White's "Flora of the Hermit shale" 109
Revision of Chicago Textbook 332
Rhizopus, effect of metals on growth of 183
Ridley, H. N., "Dispersal of plants" 335
Roses, tetraploid 55

S

Salix, nitrogen in regeneration of 320
Sap ascent 395
Schröter, C., work of 222
Selaginella, tissues of 452
Self-sterility and hybrid sterility 334
Setting of fruit in pecan 144
Sherff, E. E. 308, 465
Shull, C. A. 334, 395
Smith, Cornelia M. 377
Smith, Fanny 395
Soil, physical properties of 465; reaction and plant distribution 22
Solubility changes of inorganic constituents in citrus cuttings 213
Soy bean, floral organs of 97; nodule development of 115
Stadler, L. J., work of 111
Sterigmata in Coprinus 194
Strasburger's Textbook of botany 220

T

Temperature changes and respiration of shoot 167
Tetraploid roses of central Oregon 55
Thom, C., "The Penicillia" 109
Turesson, G., work of 336

V

Vaucheria, cell wall of 219
Vessel segment 88
Vokes, Margaret M. 194

W

Wangerin, W., work of 222
Wheats resistant and susceptible to Puccinia 1
White, D., "Flora of the Hermit shale" 109

X

X-ray mutations 110
Xylem of dicotyledons 88